Multi-centre study of neuroanatomical abnormalities in individuals at Ultra High Risk of Psychosis

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Abstract

Individuals experiencing prodromal symptoms of psychosis have a very high risk of developing the disorder ranging from 18%-36% within three years of the first clinical presentation. Currently, it is not possible to predict which individuals will subsequently become psychotic only on the basis of their presenting clinical features. This potentially prevents the selective delivery of specialised clinical interventions to those individuals more likely to develop psychosis, which is desirable, both from an ethical point of view and for a more targeted use of available treatments. Neuroimaging may aid prediction as recent neuroimaging studies suggest that there are neuroanatomical differences in people at ultra high risk (UHR) for psychosis relative to healthy control subjects. Furthermore, within UHR cohorts, those who later develop a psychotic disorder (UHR-T, transition) often show more marked structural alteration than those that do not (UHR-NT, non-transition). However the findings have been inconsistent and this may partly reflect the use of small samples and different analytic methods. The aim of this doctoral project was to assess brain structure in individuals at UHR of psychosis using a larger sample than in previous studies. This was achieved by combining Magnetic Resonance Imaging (MRI) data from four different scanning sites and using a range of different analytic methods including voxel-based morphometry, voxel-based cortical thickness analysis and multivariate machine learning. The use of these methods allowed a comprehensive investigation of neuroanatomical differences in a large cohort and, between UHR-T and UHR-NT cases in terms of i) regional gray matter volume; ii) cortical thickness; and iii) subtle and distributed patterns of gray matter alterations. Findings suggest that there are neuroanatomical abnormalities that precede the emergence of psychosis within a distributed fronto-temporal network. In addition, UHR and healthy controls are distinguishable at the individual level based on information on the gray matter volume, whereas UHR-T and UHR-NT are distinguishable at the individual level using cortical thickness information. Nevertheless, the accuracies reported remain relatively low to be applied in real-world clinical settings. Results from this project contribute to expanding the available knowledge on the UHR population.

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Mechelli A., Riecher-Rossler A., Meisenzahl E.M., Tognin S. (corresponding author), Wood S.J., Borgwardt S.J., Koutsouleris N., Yung A.R., Stone J.M., Phillips L.J., McGorry P.D., Valli I., Velakoulis D., Woolley J., Pantelis C., McGuire P. (2011). Neuroanatomical abnormalities that predate the onset of psychosis: a multicenter study. Archives General Psychiatry 68: 489-95

Tognin S., Riecher-Rossler A., Meisenzahl E.M., Wood S.J., Hutton C., Borgwardt S.J., Koutsouleris N., Yung A.R., Allen P., Phillips L.J., McGorry P.D., Valli I., Velakoulis D., Nelson B., Woolley J., Pantelis C., McGuire P., Mechelli A. (2014). Reduced parahippocampal cortical thickness in subjects at ultra-high risk for psychosis. Psychological Medicine 44: 489-98

Tognin S., Pettersson-Yeo W., Valli I., Hutton C., Woolley J., Allen P., McGuire P., Mechelli A. (2014). Using structural neuroimaging to make quantitative predictions of symptom progression in individuals at ultra-high risk for psychosis. Frontiers in Psychiatry 4: 187

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1. Introduction

1.1. Ultra High Risk for psychosis

Psychotic disorders like schizophrenia have an estimated annual incidence of between 9.2 and 15.2 per 100,000, although the incidence rate might vary depending on geographic location and socio demographic background (Kirkbride, Fearon et al. 2006, McGrath, Saha et al. 2008, van der Werf, Hanssen et al. 2014). Schizophrenia is a severe disabling psychotic disorder that affects approximately 1% of the worldwide population and carries devastating effects on the individual, their carers and on wider society (van Os and Kapur 2009). This illness is characterised primarily by positive symptoms (including hallucinations and delusions), negative symptoms and alterations of volition (including social withdrawal and lack of motivation), cognitive decline (including difficulties in memory, attention and executive functioning) and affective dysregulation (van Os and Kapur 2009). Despite several advances in biological psychiatry over the past half a century, the pathophysiology of schizophrenia is not fully understood. The neurodevelopmental model of psychosis postulates that the illness is the end state of abnormal neurodevelopmental processes that started years before the illness onset (Murray and Lewis 1987, Weinberger 1987, Rapoport, Giedd et al. 2012). In line with this model, there is increasing interest in the initial phases of psychotic illness and particularly the phase that precedes the first full-blown psychotic episode. A better understanding of early and prodromal phases may help identify factors associated with vulnerability and the development of psychosis.

The first psychotic episode typically arises around late adolescence and early adulthood (Hafner, Maurer et al. 1993, Kirkbride, Fearon et al. 2006). The period that precedes the first psychotic episode is retrospectively called the “prodromal phase”. During this phase, which can last days, months or even years, the individual usually experiences a progressive decline in social and occupational functioning together with

the emergence of attenuated psychotic symptoms (Yung, Phillips et al. 2004). The symptoms experienced by these individuals are qualitatively similar to those experienced by patients with psychosis although they are less severe and less frequent. Individuals in this stage are said to have an "at risk mental state" (ARMS) and therefore to be at "ultra high risk" (UHR) of developing psychosis (Yung, Phillips et al. 1998, Yung, Phillips et al. 2004). Not all individuals presenting with these characteristics will develop a psychotic disorder. Results from a recent meta-analysis suggest that only about 18%-36% of UHR populations will develop a psychotic disorder within three years of the first clinical presentation, while the remainder will not (Fusar-Poli, Bonoldi et al. 2012).

In the past two decades increased research efforts have been directed towards the study of the prodromal phase that precedes the first episode of full-blown psychotic illness. This phase is of particular interest as it offers a unique window to explore the mechanisms underlining disease onset in the absences of confounding factors such as antipsychotic medication and illness chronicity. In particular, investigations during the prodromal phase can identify potential biomarkers and factors associated with illness progression. These data could one day be used to identify those individuals who will develop a psychotic disorder and those who will not when they first present to the clinical services. This would allow a more efficient use of the clinical resources available within early intervention services for psychosis. Moreover, there is now compelling evidence that antipsychotic medication, in addition to the well-known side effects, may also have a long-term impact on brain structure (Ho, Andreasen et al. 2011) and function (Fusar-Poli, Broome et al. 2007). This provides an additional ethical need for trying to identify individuals that will and will not develop the disorder at the earliest possible stage. Identifying biological markers that can predict the transition to psychosis is also important in light of the recent development of the staging model (McGorry, Purcell et al. 2007, Agius, Goh et al. 2010). Specifically, the identification of such biomarkers would

allow a more accurate definition of the different stages of risk and therefore would allow for more targeted, earlier, safer and more effective intervention (McGorry, Purcell et al. 2007). Stage-specific interventions might prevent or delay the progression to a later stage or facilitate the regression to an earlier stage.

Two approaches have been adopted to define individuals at increased risk for psychosis: genetically, due to a positive family history of psychosis (e.g. Edinburgh High Risk Study (Hodges, Byrne et al. 1999)) or clinically due to the presence of psychotic-like symptoms (e.g. Personal Assessment and Crisis Evaluation - PACE - clinic in Melbourne, (Yung, Yuen et al. 2005)). Both approaches have been successfully used in the past two decades, however, the genetic high-risk approach appears only to identify a relatively small (10-20%) proportion of individuals that will go on to develop psychosis and it is associated with a potentially large time gap between the subject entry to the study and the transition to psychosis (Johnstone, Ebmeier et al. 2005). The clinical high-risk approach focuses on the presence of attenuated positive symptoms of psychosis and does not require the presence of genetic vulnerability although this is included among the screening criteria (Yung, Yuen et al. 2005). The advantage of this approach is the relatively higher number of expected transitions to psychosis. The first studies that employed these criteria reported transition rates of 40% at 6 months (Yung, Phillips et al. 1998); 40.8% (Yung, Phillips et al. 2003) and 34.6% (Yung, Phillips et al. 2004) at one-year follow-up. However, transitions rates seem to have decreased since these initial studies. A recent meta-analysis estimated that the average rate for transition was 18% after 6 months, 22% after one year and up to 36% after 3 years (Fusar-Poli, Bonoldi et al. 2012). Several reasons have been suggested to explain the decrease in transition rate. With the increased awareness around the high risk syndrome and availability of early intervention services it is plausible that the decrease in transition rates might be due to: i) a reduction in the duration of untreated symptoms in patients prior to receiving help and therefore

improved long-term outcome, ii) an improvement in the effectiveness of treatments provided, or iii) more "not truly" at-risk individuals being included increasing the number of false positives (Yung, McGorry et al. 2007).

Two main sets of criteria have been used to define the clinically high-risk individuals: the "at risk mental state" (ARMS) or "ultra high risk" (UHR) and the "basic symptoms" (BS) criteria. The UHR criteria mainly focus on attenuated positive symptoms and presumably identify individuals in the "late" prodromal phase. The basic symptoms criteria mainly focus on phenomenological disturbances that are usually present at very early stages prior to the onset of psychosis and presumably identify individuals in an "early" prodromal phase (Schultze-Lutter, Klosterkotter et al. 2014). The UHR inclusion criteria represent a combination of genetic/trait and state risk factors and they require the presence of one or more of the following: attenuated psychotic symptoms (APS), brief limited intermittent psychotic symptoms (BLIPS) lasting up to seven days and resolving spontaneously, without the use of antipsychotic medication, or trait vulnerability (family history or schizotypal personality) plus a marked decline (i.e. 30%) in psychosocial functioning over the past 12 months (Genetic Risk and Deterioration Syndrome: GRD), (Yung, Yuen et al. 2005). In particular, UHR individuals can meet the GRD criteria either because of a positive family history for psychosis or/and because they meet criteria for having schizotypal personality disorder (Yung, Yuen et al. 2005). A number of studies investigating vulnerability to psychosis have indeed focused on patients with schizotypal personality disorder (SPD) but also healthy individuals with high levels of psychometric schizotypy (Raine, Reynolds et al. 1994, Raine, Lencz et al. 2002, Modinos, Mechelli et al. 2010, Ettinger, Mohr et al. 2015). Individuals with SPD or high level of psychometric schizotypy are thought to share genetic, phenomenological and cognitive features with patients with schizophrenia, although the observed deficits are milder than those in patients (Raine, Reynolds et al. 1994, Siever,

Koenigsberg et al. 2002, Ettinger, Mohr et al. 2015). Although the UHR and schizotypy constructs partially overlap, individuals with SPD or high level of psychometric schizotypy seem to be associated with a relatively lower transition rate (i.e. around 5%,(Chapman, Chapman et al. 1994)) compared to UHR individuals (Fusar-Poli, Bonoldi et al. 2012). In addition when screening individuals with psychometric schizotypy or SPD there is no age restriction as there is with clinically UHR individuals (i.e. around 35 years old, (Yung, Phillips et al. 1998)). Individuals have to meet at least one of the UHR criteria during the 12 months prior to the screening assessment. On the other hand, basic symptoms are subjective disturbances of thought processing, language and attention that are distinct from classical positive psychotic symptoms, in that they are independent of abnormal thought content (Schultze-Lutter 2009). The different instruments for the screening of UHR individuals used by the four centres involved in this study will be discussed in details in Chapter 2 (**Table 2.1** and section 2.2.).

There are several challenges that complicate the identification of individuals at ultra high risk for psychosis. In particular, prodromal symptoms overlap considerably with other psychiatric conditions such as bipolar disorder (Ellison-Wright and Bullmore 2010), personality disorders (Nitzburg, Malhotra et al. 2014, Tschoeke, Steinert et al. 2014), but also with psychotic-like experiences that might occur in the non-psychiatric population (van Os, Hanssen et al. 2000, van Os, Linscott et al. 2009). The prevalence of psychotic-like symptoms in the general population is estimated to be around 5% (van Os, Linscott et al. 2009). This means that the majority of people reporting psychotic-like experiences do not go on to develop symptoms that require clinical interventions (van Os et al., 2009). For some individuals, however, psychotic-like experiences may gradually increase in severity and frequency and become associated with distress, particularly in the context of mood and anxiety disturbances (Fusar-Poli, Nelson et al. 2014). To date, it is not possible to identify individuals who will go on

and develop frank psychosis based only on clinical observations. A recent study provides further evidence supporting the observation that clinical impression is insufficient for predicting psychosis outcome (Nelson 2010). Therefore, it is critical to develop alternative tools that may inform clinical assessment, particularly the identification of ultra high risk individuals that are most likely to develop psychosis, remain functionally impaired and/or show symptoms progression overtime. The importance is twofold; first, it would allow a more efficient use of psychological and pharmacological treatments in the UHR population; second, it would shed light on the neurobiological changes that precede frank psychosis.

1.2. Structural brain abnormalities in UHR for psychosis

As mentioned above, to date it is not possible to distinguish individuals who will subsequently develop psychosis only based on their clinical presentation. Neuroimaging offers a promising translational tool for the characterization of brain abnormalities in individuals at UHR for psychosis and of the potential biomarkers that might allow the identification of individuals who will go on to develop psychosis. Consistent with this notion, a growing number of studies using structural Magnetic Resonance Imaging (MRI) have identified neuroanatomical differences between individuals at UHR and healthy controls, and between UHR who subsequently did and did not develop psychosis. In the following sections an overview of the neuroimaging studies in UHR is provided.

1.2.1. Gray matter abnormalities in UHR for psychosis compared to healthy controls

Structural MRI has revealed a number of neuroanatomical differences at first clinical presentation between UHR individuals and healthy controls. In whole-brain voxel-based morphometry (VBM) studies, UHR subjects, regardless of clinical outcome, showed reduced gray matter volume in the frontal lobe and lateral (Meisenzahl, Koutsouleris et al. 2008) and

medial temporal regions (Borgwardt, Riecher-Rossler et al. 2007, Meisenzahl, Koutsouleris et al. 2008). Studies that used a region of interest (ROI) approach reported both gray matter volume increases (Buehlmann, Berger et al. 2010) and reductions (Phillips, Velakoulis et al. 2002) in the hippocampus, reductions in the planum polare/temporale, insula and superior temporal gyrus (Takahashi et al. 2009, 2010), increases in the pituitary volume (Garner, Pariante et al. 2005, Buschlen, Berger et al. 2011), and reductions in the anterior cingulate cortex (ACC) (Rothlisberger, Riecher-Rossler et al. 2012) in the UHR group compared to healthy controls. However, other ROI studies did not find any significant differences between healthy controls and UHR subjects in the medial temporal lobe (Wood, Yucel et al. 2005, Velakoulis, Wood et al. 2006).

Neuroanatomical alterations in psychosis may be expressed not only in terms of alterations in gray matter volume or density but also as changes in regional cortical thickness (Narr, Bilder et al. 2005, Narr, Toga et al. 2005, Fornito, Yucel et al. 2008, Venkatasubramanian, Jayakumar et al. 2008, Schultz, Koch et al. 2010) and the degree of cortical thickness asymmetry (Haller, Borgwardt et al. 2009). In line with this notion, UHR individuals have reduced cortical thickness in the prefrontal, ACC, inferior parietal, superior temporal and parahippocampal cortices relative to healthy controls (Jung, Kim et al. 2011). A more recent study also reports increased cortical thinning over time in the left middle temporal gyrus in UHR individuals compared to healthy controls (Ziermans, Schothorst et al. 2012).

1.2.2. White matter abnormalities in UHR for psychosis compared to healthy controls

A few studies have investigated white matter abnormalities in the UHR population using either VBM or diffusion tensor magnetic resonance imaging (DTI). A VBM study reported that UHR patients showed significantly lower white matter volume in the right superior temporal

lobe compared to healthy controls (Witthaus, Brune et al. 2008). Another study investigating brain volumes (i.e. total brain, gray matter, white matter, cerebellum and ventricles) and brain density reported that UHR individuals showed a smaller increase in total white matter volume over time compared to healthy controls. Post hoc analyses indicated a more pronounced decrease over time in total white matter volume in UHR individuals who developed psychosis relative to controls, and a greater decrease in total brain volume than individuals who were not psychotic. In addition, the same authors reported no gray or white matter density differences between groups at baseline, follow-up or over time (Ziermans, Schothorst et al. 2012).

With respect to DTI, a number of studies have compared individuals at UHR against healthy controls (Peters, de Haan et al. 2008, Karlsgodt, Niendam et al. 2009, Peters, Schmitz et al. 2009, Bloemen, de Koning et al. 2010, Peters, Dingemans et al. 2010, Carletti, Woolley et al. 2012, von Hohenberg, Pasternak et al. 2014). Two studies reported a reduction of fractional anisotropy (FA), an index of white matter integrity, in the white matter of the frontal lobe (Peters, Schmitz et al. 2009, Bloemen, de Koning et al. 2010), while another found a reduction in the superior longitudinal fasciculus (SLF) (Karlsgodt, Niendam et al. 2009). A longitudinal DTI study (Carletti, Woolley et al. 2012) reported relatively widespread reductions in FA the splenium and body of the corpus callosum, the inferior and SLF, the inferior fronto-occipital fasciculus. Further reductions were also observed in the external capsule, the internal capsule and the posterior corona radiate (Carletti, Woolley et al. 2012). Another recent study observed increased mean diffusivity (MD), a measure of white matter integrity, in several clusters in the right hemisphere including the superior longitudinal fasciculus, posterior corona radiata, and corpus callosum (von Hohenberg, Pasternak et al. 2014). The authors also observed an increased radial diffusivity (RD), another measure of white matter integrity, in the posterior parietal lobe (von Hohenberg, Pasternak et al. 2014). Two studies reported no

significant differences in white matter between UHR and healthy controls (Peters, de Haan et al. 2008, Peters, Dingemans et al. 2010). The observed DTI results seem rather heterogeneous, possibly due to different methods used, such as tractography (Peters, de Haan et al. 2008, Peters, Dingemans et al. 2010), voxel-wise whole-brain spatial statistics (Peters, Schmitz et al. 2009, Bloemen, de Koning et al. 2010, Carletti, Woolley et al. 2012) or tract-based spatial statistics (Karlsgodt, Niendam et al. 2009, von Hohenberg, Pasternak et al. 2014); and differences in sample size.

1.2.3. Gray matter differences in UHR-T compared to UHR-NT

Structural MRI has revealed a number of neuroanatomical differences at first clinical presentation between individuals who subsequently made transition to psychosis (UHR-T) and those who did not (UHR-NT). In whole-brain VBM studies, UHR-T relative to UHR-NT subjects showed reduced gray matter volume of the medial and lateral temporal cortex, anterior cingulate cortex (ACC), insular, inferior and superior frontal cortices (Borgwardt, McGuire et al. 2008), and reduced gray matter density of the left temporal lobe and right cerebellum (Job, Whalley et al. 2005). While the above studies used a cross-sectional design, a number of investigations have employed a within-subject longitudinal design to examine the neuroanatomical changes that occur in individuals at UHR around the time of illness onset. These studies report progressive reductions in the gray matter volume of the orbitofrontal and cerebellar cortices (Pantelis, Velakoulis et al. 2003, Borgwardt, McGuire et al. 2008), fusiform and parahippocampal cortices and cingulate gyrus (Pantelis, Velakoulis et al. 2003); superior frontal, inferior temporal, superior parietal cortices and precuneus (Borgwardt, McGuire et al. 2008) in UHR-T compared to UHR-NT.

In addition, VBM studies employing an ROI approach indicated that individuals who subsequently made transition to psychosis showed reduced volume in the bilateral insula (Takahashi, Wood et al. 2009) and

the left ACC (Rothlisberger, Riecher-Rossler et al. 2012), and increased volume of the pituitary gland (Garner, Pariante et al. 2005, Buschlen, Berger et al. 2011) and the hippocampus (Buehlmann, Berger et al. 2010). A recent VBM investigation has also shown that, in individuals at UHR for psychosis, lower scores on a semantic fluency task, which is a measure of executive functioning, are associated with reduced gray matter density in a distributed network including the right superior/middle temporal gyrus, the right insula and the left ACC, suggesting that the combination of these two types of data could inform outcome prediction in this population (Meijer, Schmitz et al. 2011). In addition, using an ROI approach, within-subject studies have found greater progressive reductions in the gray matter volume of the insula (Takahashi, Wood et al. 2009), planum polare, planum temporale, and caudal region (Takahashi, Wood et al. 2009) in UHR-T compared to UHR-NT. Other studies found a decrease in hippocampal volume over time and no differences in pituitary volume both at the follow-up time point and over time in individuals at UHR independently of clinical outcome (Walter, Studerus et al. 2012, Walter, Studerus et al. 2014). Using Cortical Pattern Matching, an advanced brain warping technique that allows extracting information on cortical gray matter density and thickness, Sun and colleagues (2009) have also revealed brain surface contraction in the prefrontal cortex in UHR-T compared to UHR-NT (Sun, Phillips et al. 2009). With regard to cortical thickness, UHR-T compared to UHR-NT individuals presented with cortical thinning of the ACC (Fornito, Yung et al. 2008). However, a few ROI studies did not find any significant differences in cortical thickness between individuals who subsequently did and did not make conversion to psychosis (Yucel, Wood et al. 2003, Velakoulis, Wood et al. 2006, Takahashi, Wood et al. 2009, Buehlmann, Berger et al. 2010, Hannan, Wood et al. 2010, Wood, Kennedy et al. 2010). Finally, a longitudinal study investigating cortical thickness reported progressive thinning of the anterior cingulate, precuneus and temporo-parieto-occipital cortex in UHR-T compared to UHR-NT and healthy controls (Ziermans, Schothorst et al. 2012).

1.2.4. White matter differences in UHR-T compared to UHR-NT

A small fraction of studies of the UHR population have investigated white matter abnormalities associated with transition to psychosis using either VBM or DTI. Only two studies have investigated white matter abnormalities in UHR subjects as a function of clinical outcome using VBM (Walterfang, McGuire et al. 2008, Ziermans, Schothorst et al. 2012). In the first study, UHR-T subjects showed increased white matter volume in the left frontal lobe and a progressive decrease in the left fronto–occipital fasciculus (Walterfang, McGuire et al. 2008). In the second study, UHR-T subjects showed a decrease in total white matter volume relative to healthy controls but not relative to UHR-NT, in addition to which the comparison between UHR-NT and controls was also not significant (Ziermans, Schothorst et al. 2012). Using DTI, three studies have subdivided the UHR group according to clinical outcome (Karlsgodt, Niendam et al. 2009, Bloemen, de Koning et al. 2010, Carletti, Woolley et al. 2012). One of these studies revealed that UHR-T had lower FA at baseline compared to healthy controls in the medial frontal region (Bloemen, de Koning et al. 2010). In addition, UHR-T had lower FA in the white matter lateral to the right putamen and in the left superior temporal gyrus but higher FA in the left posterior temporal white matter, compared to UHR-NT (Bloemen, de Koning et al. 2010). Finally, in UHR-T, the FA in the left middle temporal lobe was negatively associated with the severity of positive symptoms (Bloemen, de Koning et al. 2010). The remaining two studies reported no cross-sectional differences in white matter integrity between UHR-T and UHR-NT (Karlsgodt, Niendam et al. 2009, Carletti, Woolley et al. 2012). However, Carletti and colleagues (2012) reported a progressive FA reduction in the left frontal white matter in UHR-T, which was not evident in UHR-NT (Carletti, Woolley et al. 2012).

1.3. Pattern classification and UHR for psychosis

Taken collectively, the above studies provide evidence for regional neuroanatomical alterations in individuals at ultra high risk for psychosis

relative to healthy controls, most evidently in a distributed network that includes lateral and medial temporal structures, prefrontal and cingulate regions, insula, cerebellum, frontal and temporal white matter. It should be noted that the above results were based on univariate statistical analyses that are sensitive to effects that are robust and clearly localised; however, it is possible that neuroanatomical changes in psychiatric disorders (including psychosis) may be subtle and distributed throughout the brain. In addition, the above results were based on average group-differences, and therefore do not allow for the characterization at the level of the individual. In day-to-day clinical practice, however, the clinician is required to make individual assessments and treatment decisions about individual patients. In recent years, an increasing number of studies have therefore adopted alternative analytical approaches based on multivariate machine learning (Orru, Pettersson-Yeo et al. 2012, Pettersson-Yeo, Benetti et al. 2013). Multivariate machine learning methods allow one to make predictions that are specific to a given individual, rather than providing an average estimate for a group. This greatly increases the translational potential of the results in a real word clinical setting. For example, Support Vector Machine (SMV) allows for the simultaneous comparison of all brain voxels with the advantage of being able to take into account the inter-relationship between voxels, detect subtle and distributed differences across the brain and make inference at the individual-level (Lao, Shen et al. 2004, Noble 2006, Norman, Polyn et al. 2006). Recent studies using multivariate methods report high levels of accuracy in the prediction of group membership, effectively distinguishing between UHR and healthy controls, between UHR-T and UHR-NT subjects (Koutsouleris, Meisenzahl et al. 2009, Koutsouleris, Borgwardt et al. 2012) and between UHR-T and first episode psychosis (Borgwardt, Koutsouleris et al. 2013). For instance, Koutsouleris and colleagues (2009) using volumetric analysis of structural MRI have successfully distinguished UHR-T group from HC group with an accuracy of 94%, UHR-NT from HC with an accuracy of 86% and UHR-T from UHR-NT with

an accuracy of 82% (Koutsouleris, Meisenzahl et al. 2009). Similar results from an independent data set study showed classification accuracy respectively of 92.3% for healthy controls and UHR-T, of 66.9%, for healthy controls and UHR-NT and of 84.2%, for UHR-T and UHR-NT (Koutsouleris, Borgwardt et al. 2012). The same authors combined MRI data from two early intervention services and transition outcome was correctly predicted in 80% of the test cases (Koutsouleris, Riecher-Rossler et al. 2014). Finally, in a recent study, UHR-T individuals have been successfully distinguished from those who experienced a first episode of psychosis with an accuracy of 80% (Borgwardt, Koutsouleris et al. 2013).

The majority of the studies employing multivariate machine learning methods in the UHR population have focused on prediction of group membership (e.g. UHR/HC) or clinical outcome in terms of transition/non-transition to psychosis. However follow-up studies of individuals at UHR have shown heterogeneity both in terms of symptoms progression and level of functioning independently of clinical outcome (i.e. transition/non-transition). For example, a recent study reported that after three years form the first presentation about 75% of UHR individuals that do not develop a psychotic disorder also show symptom remission and no longer meet UHR criteria. Conversley, 25% of cases still present with sub-threshold symptoms (Velthorst, Nieman et al. 2011). However, another study reported that some of those who present complete or partial symptomatic remission remain at a lower level of functioning when compared to a non-psychiatric population (Addington, Cornblatt et al. 2011). A further study reported that only 30% of those who do not develop psychosis meet full symptomatic and functional remission (Schlosser, Jacobson et al. 2012). Recent studies have applied other multivariate machine learning techniques such as Relevance Vector Regression (RVR) to make quantitative predictions of a variable of interest (i.e. patient's score on a clinical scale). This technique has been applied in several neuroimaging studies of healthy individuals (Franke,

Ziegler et al. 2010, Mwangi, Hasan et al. 2013), of patients with psychiatric (Mwangi, Matthews et al. 2012, Gong, Li et al. 2014) and neurological disorders (Stonnington, Chu et al. 2010). However, this technique has never been employed to make quantitative predictions in the UHR population.

In conclusion, to date multivariate machine learning methods provided promising results based on a relatively small group of subjects; to evaluate their translational applicability in real world clinical settings, however, more evidence must be collected in a larger and more representative group of subjects. In addition, although these methods have been successfully employed to make predictions about group membership and clinical outcome in people at UHR for psychosis, they have yet to be employed to make quantitative prediction of symptoms progression in this population.

1.4. Multi-centre approach for the study of UHR individuals

Individual structural imaging studies often report contrasting findings. This inconsistency might be partially explained by the characteristics of the specific samples investigated. For example different research centres might use different inclusion criteria and therefore have relatively different clinical samples. Although the transition rate associated with the different screening criteria is very similar (Fusar-Poli, Bonoldi et al. 2012), it cannot be excluded that the use of different semi-structured interviews can result in the recruitment of individuals with relatively distinctive clinical profiles. The individuals recruited using different diagnostic tools might also be in a different stage of risk. According to the "early" versus "late" prodrome model proposed by the German Research Network on Schizophrenia (GRNS), individuals meeting GRD criteria and subjects experiencing "basic symptoms" are thought to be at a lower risk stage; while individuals meeting APS and BLIPS criteria are thought to be at a higher risk stage. Consistent with this model, in a recent study, Nelson and colleagues (Nelson, Yuen et al. 2011) have investigated the

different degree of risk associated to the UHR criteria. Based on this study UHR inclusion criteria predict transition over six months in the order of GRD group alone, APS group and BLIPS group. These differences in transition risk associated with distinct UHR criteria might reflect different underlying neurobiological features. In addition, the samples might differ in age, gender, genetic vulnerability, co-morbidities, substance use and medication use. Another possible source of variability across different studies relates to the relatively small sample size (Button, Ioannidis et al. 2013) as it is very difficult for a single centre to recruit a relatively large number of UHR individuals. Sample size is a particular problem for the comparison between UHR subjects who later develop psychosis and those who do not, which entails a further subdivision of the UHR sample according to clinical outcome. Lastly, past studies have used a wide range of analytical techniques to investigate structural abnormalities and this further complicates results comparisons.

A way to overcome these complications, to improve statistical power and the generalizability of the results is to use a multi-centre approach, with the pooling of data to produce a relatively large total sample. This approach, which has been successfully employed in neuroimaging studies of other CNS disorders in which subject recruitment is difficult (Nestor, Rupsingh et al. 2008, Stonnington, Tan et al. 2008), was used in the present doctoral work.

1.5. Outline and objectives of the studies

1) Are there neuroanatomical brain differences between UHR and healthy controls?

My first objective was to use voxel-based morphometry (VBM) and voxel-based cortical thickness (VBCT) measurements to test whether there are volumetric and cortical alterations in UHR cohorts that can help to distinguish them form healthy controls. I aimed to investigate whether these analytical techniques are also able to provide complementary

information depending on the underlying pathophysiological mechanisms. This part of my doctoral work is described in Chapters 3 and 4.

2) Are there neuroanatomical brain differences between UHR-T and UHR-NT?

My second objective was to use VBM and VBCT measurements to test whether there are volumetric and cortical alterations that can help to distinguish between UHR-T and UHR-NT. I aimed to investigate whether these analytical techniques are also able to provide complementary information depending on the underlying pathophysiological mechanisms. This part of my doctoral work is described in Chapters 3 and 4.

3) Can brain structure information be used to aid the identification of UHR individuals?

My third objective was to use a multivariate pattern classification technique, Support Vector Machine (SVM), to distinguish between UHR and HC, using structural imaging data (i.e. volume and cortical thickness). This part of my doctoral work is described in Chapter 5.

4) Can brain structure information be used to aid the identification of those individuals who will make transition to psychosis (UHR-T)?

My fourth objective was to use a multivariate pattern classification technique, SVM, to distinguish between UHR-T and UHR-NT, using structural imaging data (i.e. volume and cortical thickness). This part of my doctoral work is described in Chapter 5.

5) Can brain structure information be used to predict symptom progression in UHR individuals?

My fifth objective was to use a multivariate pattern classification technique, Relevance Vector Regression (RVR), to make quantitative prediction of symptom progression in UHR, using structural imaging data

(i.e. volume and cortical thickness). This analysis was performed in a single centre where more detailed follow-up information was available. This part of my doctoral work is described in Chapter 6.

2. Methods

2.1. Study sample

In the past two decades increased efforts have been directed to the identification of the individuals experiencing possible prodromal signs of psychosis in order to isolate factors that are associated with the development of this illness. Several approaches have been used to identify people at increased risk of psychosis. One approach has applied the genetic risk criteria identifying individuals that have a first-degree relative with a psychotic disorder (Hodges, Byrne et al. 1999). This approach however is associated with relatively low predictability, since 10-20% of individuals who meet these criteria go on to develop psychosis (Johnstone, Ebmeier et al. 2005). Another approach has focused on young individuals who are experiencing the emergence of "basic symptoms". Basic symptoms are described as subjective changes in cognitive, emotional, motor and autonomic function, as well as in bodily sensations, external perceptions, and tolerance to normal stress (Schultze-Lutter, Klosterkotter et al. 2014). Approximately 70% of individuals experiencing basic symptoms will develop a psychotic disorder in the following 10 years (Klosterkotter, Hellmich et al. 2001). A further approach has employed a strategy that combines state and trait risk factors. This "close-in" strategy focuses on individuals in the age of maximum incidence of the disorder and includes the screening of psychotic-like symptoms and positive family history (Yung, Phillips et al. 1998).

Three semi-structured interviews have been developed for the assessment of young individuals at high risk of psychosis: the

Comprehensive Assessment of At-Risk Mental States (CAARMS), the Structured Interview for Prodromal Syndromes (SIPS) and the companion Scale of Prodromal Symptoms (SOPS), and the Basel Screening Instruments for Psychosis (BSIP). The CAARMS was developed by Yung and colleagues (Yung, Yuen et al. 2005) at the Personal Assessment and Crisis Evaluation (PACE) clinic in Melbourne and has been used in Australia, Asia and Europe. The SIPS/SOPS have been developed by McGlashan and colleagues and have been mostly used in North American studies (McGlashan, Zipursky et al. 2003, Miller, Zipursky et al. 2003). The BSIP has been developed by Riecher-Rossler and colleagues in the FEPSY clinic in Basel (Riecher-Rossler, Aston et al. 2008). Interestingly, the "attenuated psychotic symptoms" as defined by the CAARMS (see Table 2.1.) are partly overlapping with the Chapman's scales (Chapman, Edell et al. 1980, Eckblad and Chapman 1983) and the Rust Inventory of Schizotypal Cognition (RISC), (Rust 1988), that measure schizotypal personality variation. This suggests the possible presence in the UHR clinical sample of people also presenting with schizotypal personality traits. In addition, two instruments have been developed for the assessment of basic symptoms: the Bonn Scale for the assessment of Basic Symptoms (BSABS), (Gross 1987), and the Schizophrenia Proneness Instrument, Adult Version (SPI-A), (Schultze-Lutter 2007), both used in Europe. These instruments assess self-perceived cognitive and perceptual changes. Details relative to the different instruments are reported in **Table 2.1.**

Table 2.1. Assessment Instruments for clinical high risk samples

Criteria	CAARMS	SIPS/SOP	BSABS/SPI-A
Attenuated Psychotic Symptoms (APS)	Subthreshold attenuated positive symptoms: e.g. ideas of reference, 'magical' thinking, perceptual disturbance,	Subthreshold attenuated positive symptoms: e.g. unusual ideas, paranoia/suspiciousness, grandiosity, perceptual disturbance,	

	paranoid ideation, odd thinking and speech; held with either subthreshold frequency or subthreshold intensity; present for more than 1 week within the past year and for less than 5 years	conceptual disorganization; without psychotic level conviction; onset or worsening in the past year; frequency: at least once per week in the past month	
Brief (limited) Intermittent Psychotic episode (BIP/BLIP)	Transient psychotic symptoms: symptoms in the realm of delusions, hallucinations, disorganization; duration of the episode < 1 week; spontaneous remission; symptoms occurred within 1 year but for not longer than 5 years	Transient psychotic symptoms: symptoms in the realm of delusions, hallucinations, disorganization; onset in past 3 months; frequency: at least 1 hour/day at min. average frequency of 4 day/week over a one month period or symptoms are seriously disorganizing/dangerous	
Genetic Risk and Deterioration syndrome (GRD)	First-degree relative with a psychotic disorder or schizotypal personality disorder, or an individual with schizotypal personality disorder and significant decrease in mental state or functioning* maintained for at least 1 month and for less than	First-degree relative with a psychotic disorder OR an individual with schizotypal personality disorder AND a significant decrease in functioning* in the past month compared to one year ago	First-degree family member with schizophrenia or ante/prenatalcomplications and significant decrease in functioning* for less than 1 month

	5 years		
Basic Symptoms (BS)			Subtle subjective disturbances of cognition and perception: at least 1 of 10 Basic symptoms with a score of at least 3 within the last 3 months and first occurrence within 1 year or cognitive disturbances : at least 2 of 9 basic symptoms with a score at least 3 within the last 3 months

Table 2.1. adapted from a published paper (Valli, Tognin et al. 2012). *Defined as a 30% drop in Global Assessment of Functioning Scale score within the last year**.** Abbreviations: APS, Attenuated Psychotic Symptoms; BS, Basic Symptoms; BIP/BLIP, Brief (limited) Intermittent Psychotic episode; BSABS, Bonn Scale for the assessment of Basic Symptoms; CAARMS, Comprehensive Assessment of the At Risk Mental State; GRD, Genetic Risk and Deterioration syndrome; SIPS/SOPS, Structured Interview for Prodromal Syndromes (Scale of Prodromal Symptoms); SPI-A, Schizophrenia proneness instrument Adult version.

2.2. Recruitment

UHR participants were recruited from four specialised clinical services for young people at risk for psychosis in London, Basel, Melbourne and Munich. Recruitment details and criteria used by the single centres are reported below. Sociodemographic characteristics of the study samples by site are reported in Appendix 3 (Table A3.1. and A3.2.).

London: All participants at UHR of developing psychosis from the London site were recruited through the Outreach and Support in South London (OASIS) service (Fusar-Poli, Byrne et al. 2013). OASIS is a community mental health service that provides support and treatment to young people aged between 14 and 35 who are considered to be at increased risk of developing psychosis. OASIS is part of the South London and Maudsley NHS Foundation Trust (SLAM; www.slam.nhs.uk), it covers

the boroughs of Lambeth and Southwark and has recently expanded to the borough of Lewisham. The service started operating in 2001 and its aims are to provide support and treatment to UHR individuals in order to prevent or delay the transition to psychosis. OASIS offers a follow-up period of two years during which clients are offered psychological interventions, pharmacological treatment if required, medical reviews, practical support with the aim of reducing symptoms, social and occupational impairment, and improving outcome if any clients go on to develop psychosis. UHR individuals were defined using PACE criteria (Yung et al. 1998) and assessed using the CAARMS (Yung, Yuen et al. 2005). UHR individuals meet PACE criteria if they display one of the following: 1) Attenuated Psychotic Symptoms (APSs) that include disorder of thought content or form, disorder of perceptions that do not reach the threshold for a DSM-IV diagnosis of psychotic disorder; 2) Brief Limited Intermittent Psychotic Symptoms (i.e. symptoms are of psychotic intensity and frequency but they do not last for more than 7 days and resolve spontaneously); 3) positive family history or schizotypal personality disorder plus a marked decline (i.e. 30%) in psychosocial functioning over the past 12 months (Genetic Risk and Deterioration Syndrome: GRD) as assessed by the Global Assessment of functioning (GAF), (Endicott, Spitzer et al. 1976).

Basel: All individuals at risk for psychosis in Basel were recruited through the FEPSY (Frueherkennung von Psychosen = early detection of psychosis) clinic (Riecher-Rossler, Gschwandtner et al. 2007). UHR individuals were assessed using the BSIP (Riecher-Rossler, Aston et al. 2008), the Brief Psychiatric Rating Scale (BPRS) (Rhoades and Overall 1988), and the Scale for the Assessment of Negative Symptoms (SANS), (Andreasen 1989). The BSIP was used to evaluate prodromal symptoms (defined according to DSM-III-R) occurring in the last 5 years; nonspecific prodromal signs (Hafner, Riecher et al. 1991) in the last 2 years; previous or current psychotic symptoms, psychosocial functioning over the last 5 years, substance dependency; and psychotic disorders

among first and second degree relatives. The UHR group was defined using criteria corresponding to the PACE criteria (Yung, Phillips et al. 1998).

Melbourne: All individuals at risk for psychosis in Melbourne were recruited through the PACE clinic which manages young people at risk of developing a psychotic illness (Yung, Phillips et al. 1998). The UHR individuals were screened according to the PACE criteria (Yung, Phillips et al. 1998, Yung, Yuen et al. 2005) and were assessed using the BPRS (Rhoades and Overall 1988), and the SANS, (Andreasen 1989).

Munich: All individuals at risk for psychosis in Munich were recruited at the Early Detection and Intervention Centre for Mental Crises, Ludwig-Maximilians-University (Meisenzahl, Koutsouleris et al. 2008). Potential UHR individuals were examined according to a standardized inclusion criteria checklist with operationalized definitions of different types of prodromal symptoms: basic symptoms taken from the BSABS (Gross 1987), attenuated psychotic (APSs) and brief limited intermittent psychotic symptoms (BLIPSs) as defined by the PACE criteria (Yung, Phillips et al. 1998).

Although the single centres have used different screening instruments, the criteria adopted to identify and assess UHR individuals were comparable across sites and corresponded to the PACE criteria (Yung, Yuen et al. 2005).

Healthy controls ages 18 to 35 (ages 16 to 35 in Melbourne), were recruited over the same period by the single centres from the same socio-demographic area through local advertisement. Healthy participants had no history of psychiatric disorder and had no first-degree relatives with a diagnosis of a psychotic illness.

2.2.1. Transition rates across sites

Potential differences in the proportion of individuals who transitioned to psychosis across the different sites were examined using a Chi-squared test. The fraction of participants who transitioned to psychosis varied from 40% to 9.5% (Table A3.1, and A3.2. Appendix 3). A chi-squared test across four centres (after removing the London - Institute of Psychiatry site that comprised of only 2 UHR-T individuals and was excluded from most of the analyses) was not significant ($\chi^2(3)=7$, p=0.072 based on the data reported in Chapter 3; $\chi^2(3)=4.259$, p=0.235 based on the data reported in Chapters 4 & 5). Therefore, although nominally different transition rates were observed in the different centres, there was no statistically significant effect of site on the percentage of those who transitioned to psychosis. The percentages of UHR individuals by site who have transitioned to psychosis are reported in Table 3.1. in Chapter 3 and in Table 4.1 in Chapter 4.

2.2.2. Inclusion and exclusion criteria

Participants in the two groups met the following criteria:

- Age 18 to 35 years old (Melbourne: 16 to 35 years old)
- No history of a neurological disorder or severe head injury
- No past or present diagnosis of schizophrenia spectrum or bipolar disorder, as well as borderline personality disorder, delirium, dementia, amnestic or other cognitive disorders, mental retardation, and psychiatric disorder due to a somatic factor, following DSM-IV criteria
- Estimated premorbid IQ greater than 70
- No evidence of alcohol or drug use meeting DSM-IV criteria for dependence disorder
- No contraindication to exposure to a magnetic field such as presence of metal implants pacemakers, and pregnancy

Participants in the healthy control group met the following additional criteria:

- No history of psychiatric disorder
- No history of a first-degree relative with a diagnosis of a psychotic disorder.

2.2.3. Ethics

Ethical approval for the study was obtained separately at the single centres.

2.2.4. Informed consent

Informed written consent was obtained from all the participants at the single centres.

2.2.5. Data protection

All data of the study were stored in compliance with the Data Protection Act 1998. Participants were referred to by study number only. Contact details were stored on a password-protected server.

2.2.6. Confidentiality

Confidentiality was discussed with all the participants at the single centres. It was stated that they would not be identifiable in any publications arising from the study and that there would not be any way of linking their identity to the study. All subjects agreed that their general practitioner would be contacted should any medically relevant information require further investigation.

2.3. Structural Magnetic Resonance Imaging

Structural MRI relies on the concept of nuclear magnetic resonance (NMR). Protons contained in atomic nuclei have a positive electrical charge and revolve around an axis. This quantum quality spin produces a magnetic field with a north-south polarity along the spin axis (the magnetic vector). In the absence of an external magnetic field, individual spins are randomly oriented and bulk material has no magnetisation. However, when an external magnetic field B_0 is applied, the individual

magnetic spins align with it or rather process around the magnetic field direction.

A spinning proton can have different energies depending on its orientation relative to the applied magnetic field B_0. MRI techniques measure the effects of changing the spin of particular atomic nuclei. In living organisms the most abundant source of protons derives form hydrogen atoms contained in water. For the simple spin system of hydrogen, the spinning nucleus can have two orientations relative to the applied magnetic field B_0. The parallel orientation is associated with a low energy state, while the anti-parallel orientation is associated with a high-energy state. The sum over all the nuclei in an object gives the net magnetisation of the object. Its description is based on a coordinate system with the *z* axis being in the direction of the applied magnetic field B_0. In the resulting magnetisation state more spins are in the low rather than in the high-energy state. Summing the contributions of singular magnetic vectors will therefore give a net magnetic vector M_0 along the direction of the applied magnetic filed. In addition, the rotating magnetisation of each nucleus has a small component projecting onto the *zy* plane, transverse to the filed direction. However, in resting conditions, opposing magnetic components of the transverse magnetisation sum to zero.

With the application of an oscillating radiofrequency electromagnetic field B_1, perpendicular to the main magnetic field B_0, spins can be excited from the low to the high-energy state. The most efficient transfer of energy occurs when the oscillating frequency of the B_1 and the frequency of the protonic spin are the same. This phenomenon is called resonance and will result in a decrease of the longitudinal field M_0. A second effect of the radiofrequency (RF) pulse B_1 is that protons are brought into coherence. Individual magnetic vectors in the transverse plane no longer cancel to zero, pointing instead in the same direction and resulting in a new magnetic vector M_1 on the transverse plane. The transverse

component of the magnetic vector determines the detectable NMR signal because it induces an electric current detected by a coil on the xy plane. The amplitude of the current depends on the proton density, with higher density determining greater magnetisation vector returning to its original position along the *z* axis (i.e. relaxation). The molecular environment is reflected in the time variation of signal amplitude as the nuclei return to equilibrium. This process is gradual and happens in two ways: a) energy can be given to neighbouring molecules in the surrounding environment (spin-lattice relaxation), and b) energy can be transferred to nearby nuclei (spin-spin relaxation).

T1 relaxation: Spin-lattice or T1 relaxation describes the re-growth of the magnetisation vector along the *z* axis, an exponential process described by the T1 time constant. Excited protons will dissipate energy to molecules of the surrounding structures (lattice) as heat and the exact composition of the environment will affect T1.

T2 relaxation: Spin-spin or T2 relaxation describes the disappearing coherence of the transversal magnetic vector M1 on the *xy* plane, which occurs at a different rate to the recovery of magnetisation along the *z* axix. The term spin-spin refers to the fact that interactions between protons determine the rate of T2 relaxation. No energy is lost, but an exchange of energy between protons causes their rotation to become desynchronised, resulting in a gradual decrease of M_1.

2.3.1. Image formation

Using a homogenous field B_0 would not produce a tomographic image because all protons would experience the same magnetic field and therefore the frequencies of their emitted signal would be identical. A non-uniform magnetic field B_1 is thus applied in order for the resonance frequencies of spins to vary within the sample. This gradient will cause protons to emit different frequency signals according to their spatial position. For each frequency component of the measured signal, the known value of the applied strength and direction of the magnetic field

can be used to calculate the position from which the signal originated. A non-uniform magnetic field is combined with a spin echo, a pulse used to dampen the loss of transversal magnetisation that is determined by the inhomogeneity of the magnetic field itself. If the signal were acquired measuring frequencies only, its spectrum would be a one-dimensional projection of spin density in the selected slice. To produce a two-dimensional image, encoding on a second axis is required. Locations are encoded by frequency on the first axis, and by phase on the second axis. Location dependent phase is achieved using further gradient on the second axis. The duration of the applied gradient dictated the degree to which local transversal magnetisations are dephased. A series of increasing pulse lengths will enable a reconstruction of the frequencies giving rise to dephasing of transversal magnetisation. Step-wise increases in both gradients divide the sample into volume-elements or voxels. Spins in one voxel experience the same frequency and phase encoding and the signal from a given voxel is the sum of all individual spin contributions. The resolution of the image depends on the size of the voxels, which is determined by the step size of the gradients.

2.3.2. Image acquisition parameters

London The MR images were acquired on a 1.5 T GE NV/I Signa LX Horyzon system (General Electric, Milwaukee, WI, USA) at Mapother House, Maudsley Hospital. T1-weighted Inversion Recovery Spoiled Gradient structural images were acquired with the following acquisition parameters: time-to-echo, 5.2 milliseconds; time-to-repetition, 15.9 milliseconds; flip angle, 20°; field of view, 220 x 200 mm; slice thickness, 1.5 mm; number of slices, 128; matrix size, 256 x 256 x 124. Another set of MR images were acquired on a 3 T GE Excite II at the Centre for Neuroimaging Sciences (CNS) using a fast Spoiled Gradient Recalled (FSPGR) with the following acquisition parameters: time-to-echo, 2.82 milliseconds; time-to-repetition, 6.96 milliseconds; flip angle, 20°; field of view, 280 x 280 mm; slice thickness, 1.1 mm; number of slices, 196; matrix size, 256 x 256.

Basel Subjects were scanned using a Siemens (Erlangen, Germany) Magnetom Vision 1.5 T scanner at the University Hospital Basel. A three-dimensional volumetric spoiled gradient recalled echo sequence generated 176 contiguous, 1 mm thick sagittal slices. Imaging parameters were: time-to-echo, 4 milliseconds; time-to-repetition, 9.7 milliseconds; flip angle, 12; matrix size, 200 x 256; field of view, 25.6 x 25.6 cm matrix; and voxel size, 1.28 x 1 x 1 mm.

Melbourne The MR images were obtained on a GE Signa 1.5 T scanner at the Royal Melbourne Hospital (RMH) and at the Royal Children Hospital. A three-dimensional volumetric spoiled gradient recalled echo in the steady state sequence generated 124 contiguous, 1.5 mm thick coronal slices. Imaging parameters were: time-to-echo, 3.3 milliseconds; time-to-repetition, 14.3 milliseconds; flip angle, 30°; matrix size, 256 x 256; field of view, 24 x 24 cm matrix; voxel dimensions, 0.937 x 0.937 x 1.5 mm.

Munich The MR images were obtained on a 1.5 T system (Magnetom Vision; Siemens, Erlangen, Germany). Imaging was performed with a T1-weighted three-dimensional magnetization pre-pared rapid-acquisition gradient echo sequence with the following acquisition parameters: time-to-repetition, 11.6 milliseconds; time-to-echo, 4.9 milliseconds; field of view, 230 mm; 512 x 512 matrix; 126 contiguous axial sections of 1.5 mm thickness; and voxel size, 0.45 x 0.45 x 1.5 mm.

The datasets collected in the four centres were combined to form a large multi-centre sample.

Chapter 3 sample. The study described in this chapter included five datasets: the two datasets from London, the one from Basel, the one from Melbourne, and the one from Munich.

Chapter 4 and 5 samples. The studies described in these chapters included four datasets: one from London (i.e. 1.5 T), the one from Basel, the one from Melbourne, and the one from Munich.

Chapter 6 sample. The study described in this chapter included only a subsample of the London dataset (i.e. 1.5 T).

2.4. Structural image analyses

Several different packages are available for the analysis of imaging data. Statistical Parametric Mapping (SPM, http://www.fil.ion.ucl.ac.uk/spm) is one of the most used. Version SPM8 implemented in MATLAB 7.1 (MathWorks, Natick, MA, USA) was employed in this doctoral work. Image analysis of structural images was performed using voxel based morphometry (VBM) and voxel based cortical thickness (VBCT). Both involved voxel-wise statistical analysis of pre-processed structural images, implemented with SPM8 running under MATLAB 7.1. VBM identifies regional differences in cerebral tissue by comparing different brains on a voxel-by-voxel base (Ashburner and Friston 2000). In order to compare different brains, all the structural images are spatially normalised to the same stereotactic space, segmented in gray and white matter and smoothed by convolution with a Gaussian kernel (Ashburner and Friston 2000). An additional 'modulation' step can be applied to preserve the total volume as opposed to concentration (Mechelli, Price et al. 2005). An alternative approach that can be employed to assess brain differences between groups is to estimate the cortical thickness. This procedure involves the identification of the inner and outer cortical surfaces. Two approaches have been employed to estimate cortical thickness: surface based and voxel-based. In surface-based approaches, image information and surface geometry are used to construct a representation of the gray and white matter surfaces. The cortical thickness is therefore estimated by computing the distance between corresponding points in the two surfaces (Fischl and Dale 2000). In voxel based approaches, such as VBCT, the gray and white matter boundaries are defined only on the basis of voxel information and therefore cortical thickness is estimated based on the trajectory between the two boundaries within each voxel (Hutton, De Vita et al. 2008).

The VBM analysis of gray matter volume returns a mixed measure that depends on cortical thickness as well as cortical folding and gyrification (i.e. cortical surface area), whereas the VBCT analysis measures the one-dimensional scalar thickness of the cortex at each voxel location (Hutton, De Vita et al. 2008). Therefore VBM and VBCT can be considered as complementary methods that can characterise different morphological aspects of the brain. Preprocessing details relative to VBM and VBCT are reported below, in section 2.4.1.1 and 2.4.1.2.

2.4.1. Image preprocessing

VBM and VBCT share the same initial preprocessing steps and voxel-wise statistical approaches. Structural images were firstly pre-processed with a unified segmentation procedure. The unified segmentation procedure (Ashburner and Friston 2005) implemented in SPM8 (http://www.fil.ion.ucl.ac.uk/spm) was used to segment all the images into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) partitions. Images were then pre-processed and analysed using the two alternative approaches (i.e. VBM and VBCT) that allowed extracting information on gray matter volume and cortical thickness respectively.

2.4.1.1. Preprocessing of gray matter volume for VBM analyses

A fast diffeomorphic image registration algorithm (DARTEL) was used to warp the gray matter partitions into a new study-specific reference space with an isotropic spatial resolution of $1.5mm^3$ (Ashburner and Friston 1997, Ashburner 2007, Ashburner and Friston 2009). The warped gray partitions were then affine transformed into the MNI space. An additional 'modulation' step (Mechelli, Price et al. 2005) was used to scale the gray matter probability values by the Jacobian determinants of the deformations to ensure that the total amount of gray matter in each voxel was conserved after the registration. As a final step the gray matter probability values were smoothed using a 8mm FWHM Gaussian kernel.

2.4.1.2. Preprocessing of cortical thickness for VBCT analyses

A voxel-based Laplacian method (Jones, Buchbinder et al. 2000, Hutton, De Vita et al. 2008) was used to create a voxel-based cortical thickness (VBCT) map for each subject using the GM, WM and CSF partitions created in the segmentation step. The resulting VBCT maps contained cortical thickness (CT) values within voxels identified as gray matter and zeros outside the cortex and were saved in the native space of the input images ($0.5mm^3$ resolution). Each VBCT map was warped into the new DARTEL reference space by applying the corresponding subject specific deformation field and resampled to an isotropic voxel size of $1.5mm^3$. The warped images were then scaled by the Jacobian determinant of the deformation and smoothed with a 6mm FWHM Gaussian kernel. The same warps, modulation and smoothing were also applied to a binary mask created from each original VBCT map. Subsequently the warped, scaled and smoothed VBCT maps were divided by the corresponding warped, scaled, and smoothed mask. The effect of this procedure was to project the Gaussian smoothing kernel applied to the warped images, into the native space of the subject while preserving the CT value over a region the size of the smoothing kernel.

2.4.1.3. Statistical analyses

The voxel-wise statistical analysis on gray matter volumes and cortical thickness was performed using the General Linear Model, a flexible framework that allows a number of different statistical tests, under the assumption that the data are described by effects of interest, confounds of no interest and residual component of error. After fitting the model to the data, parameters are estimated for each effect entered into the model. Standard parametric statistics (t-test and F-test) are then applied to the estimated parameters in order to test hypothesis about differences between effects of interest and the results are then reported as Statistical Parametric Maps (SPMs). At this stage many voxel-wise tests are computed and a correction for multiple comparisons is necessary. During the preprocessing, the images have been smoothed and gray matter in

neighbouring voxels is highly correlated, therefore a standard Bonferroni correction would not be appropriated. A correction for multiple comparisons based on Gaussian Random Fields (GRF) theory is applied to minimize the risk of false positives in a random data set (Worsley, Marrett et al. 1996).

2.4.2. Multivariate analysis of structural data

Standard mass-univariate analyses allow the identification of anatomical differences that are statistically significant at the group level; these however are of limited use in clinical practice where a clinician must make inferences at the individual level. A further characteristic of the mass-univariate approach is that it treats each voxel individually and therefore is ideally suited to identifying strong, localised differences; however it is not very sensitive to signals from different regions that are spatially correlated. A third consideration is that structural MRI data are multivariate in nature because each brain volume contains information relative to thousands or voxels. Univariate approaches that involve multiple testing and the subsequent corrections for multiple comparisons may therefore be too conservative and not sensitive enough to detect network-level alterations. Multivariate machine learning analyses have therefore been applied to neuroimaging data to investigate spatially distributed alterations in the brain (Orru, Pettersson-Yeo et al. 2012).

Machine learning is a branch of artificial intelligence that allows the automatic extraction of information from data by the use of computational and statistical algorithms. Within the field of machine learning, statistical pattern recognition focuses on the recognition of patterns and regularities in data to classify data into different categories (Bishop 2006). In the machine learning application with neuroimaging data, brain images are treated as spatial patterns and statistical learning methods are used to identify which statistical properties allow the discrimination between categories or conditions (e.g. patients vs

controls). The pattern identification process consists in two phases. In the training phase, the algorithm identifies the features that best distinguish the two populations or conditions. In the testing phase, a previously unseen subject is then attributed to one of the groups based on the algorithm previously developed. Within this general framework, there are a number of specific machine learning methods that can be applied to neuroimaging data. In the present thesis, I used two of them: Support Vector Machine (SVM) and Relevance Vector Regression (RVR).

Support Vector Machine (SVM) is a multivariate method that allows the binary classification or categorization of individual data into class or categories. In neuroimaging, the three dimensional data (i.e. MR images) are transformed into an input vector (Haynes and Rees 2006). Input data are classified into two groups based on class labels. The algorithm is then trained in order to find a hyperplane that best separates the input space. A hyperplane is an n-dimentional generalisation of a plane. This in an affine subspace of n-1 dimension that splits an n-dimensional space. In situations of high dimensionality, where dimensions (e.g. voxels) exceed the number of data points (e.g. volumes or scans), several different hyperplanes that correctly separate the data are possible. The SVM algorithm (Vapnik 1999) finds the hyperplane with the maximal separation between classes. Each hyperplane is parameterised by a weight vector *w*. The projection of each data point onto the weight vector is used to find the maximum margin, where the margin is the distance of the closest training data point to the hyperplane. The points nearest to the margin are the most important for the classification because they are the ones that define the hyperplane and they are called 'support' vectors. The vector *w* is called the classifier's weight vector and it carries the information about which variables or voxels are relevant for discriminating between groups. The weight vector can be plotted to form a discriminating map that shows the relative importance or weight of each voxel in the brain for the classification. Predictive power can be assessed over a series of characteristics such as accuracy, sensitivity (e.g.

the proportion of patients identified as having a condition), specificity (e.g. the proportion of controls identified as not having a condition), positive and negative predictive values. Statistical significance of the accuracy can be determined by permutation testing; this involves repeating the classification procedure with a different random permutation of the training group labels, and dividing the number of permutations achieving higher sensitivity and specificity than the true labels by the total number of permutations.

SVM permits the classification of individual observations into distinct groups or class (e.g. UHR-T vs UHR-NT) but cannot be used to predict continuous variables (e.g. change in a patient's score). Thus an alternative machine learning method known as Relevance Vector Regression (RVR) will also be used to allow the characterization of clinical response in terms of a continuous variable (Tipping 2001). This will allow the estimation of the value of neuroimaging data for predicting quantitative changes in clinical measures without the need for an a priori cut-off to distinguish between those who did and did not make transition.

Relevance Vector Regression (RVR) is a multivariate regression method set in fully probabilistic Bayesian framework. Under this framework, a zero-mean Gaussian prior is introduced over the model weights, governed by a set of hyperparameters, one for each weight. The most probable values for these hyperparametres are estimated from the training data, with sparseness achieved due to the posterior distribution of many of the weights peaking sharply around zero. These training vectors associated with non-zero weights are referred to as 'relevance' vectors. The optimised posterior distribution over the weights can be used to predict the target value (e.g. a patient's score on a clinical scale) for a previously unseen input vector (e.g. VCBT maps) by computing the predictive distribution (see Tipping, 2001 for a detailed description).

In the present doctoral work the toolbox Pattern Recognition for Neuroimaging Toolbox (PRoNTo; http://www.mlnl.cs.ucl.ac.uk/pronto/) was used to perform the multivariate analyses of the structural data (Schrouff, Rosa et al. 2013).

3. Neuroanatomical abnormalities that predate the onset of psychosis: a multi-centre voxel-based morphometry study

(Adapted from published paper, Appendix 1, A1.1.)

This study was published in Archives of General Psychiatry under the title "Neuroanatomical abnormalities that predate the onset of psychosis: a multi-centre study".

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3.1. Introduction

Psychotic disorders are usually preceded by a prodromal phase in which there is a gradual deterioration of global and social functioning and the emergence of attenuated psychotic symptoms (Hafner, Maurer et al. 1993, Yung, Yuen et al. 2005). However, not all people with these features go on to develop a full-blown psychotic disorder; 18-36% develop psychosis, usually within 36 months, but the remainder do not (Fusar-Poli, Bonoldi et al. 2012). Subjects presenting with this clinical syndrome are thus said to be at "ultra high risk" (UHR) of psychosis (Yung, Phillips et al. 1998). Recent trials suggest that clinical intervention in the UHR population may reduce the risk of later transition to psychosis (Amminger, Edwards et al. 2002, McGorry, Yung et al. 2003, Killackey and Yung 2007). However, it is difficult on purely clinical grounds to distinguish individuals who will later become psychotic from those who will not (McGorry, Yung et al. 2003, Riecher-Rossler, Gschwandtner et al. 2007, Riecher-Rossler, Pflueger et al. 2009). This prevents the selective delivery of potentially preventative interventions to the subgroup that are most likely to become psychotic, which is desirable both from an ethical standpoint, and in terms of the efficient use of health care resources.

Recent studies using magnetic resonance imaging (MRI) have examined whether there are neuroanatomical differences between UHR subjects who subsequently develop psychosis and those who do not (Phillips, Velakoulis et al. 2002, Job, Whalley et al. 2003, Pantelis, Yucel et al. 2003, Garner, Pariante et al. 2005, Job, Whalley et al. 2005, Velakoulis, Wood et al. 2006, Borgwardt, McGuire et al. 2007, Borgwardt, Riecher-Rossler et al. 2007, Borgwardt, McGuire et al. 2008, Fornito, Yung et al. 2008, Koutsouleris, Schmitt et al. 2009, Witthaus, Kaufmann et al. 2009, Buehlmann, Berger et al. 2010). A number of differences in regional grey matter volume have been reported, but the findings have been inconsistent; this may partly reflect the use of small samples. However UHR subjects are difficult to recruit and specialised clinical services are

usually required. Thus it is difficult for any single centre to scan a large sample. Sample size is a particular problem for the key comparison between UHR subjects who later develop psychosis and those who do not, which entails a further subdivision of the UHR sample according to clinical outcome. A potential solution is to conduct multi-centre studies, with the pooling of data to produce a relatively large total sample. This approach has been successfully employed in neuroimaging studies of other CNS disorders in which subject recruitment is difficult (Nestor, Rupsingh et al. 2008, Stonnington, Tan et al. 2008).

In the present study, magnetic resonance whole-brain images were acquired from five MRI scanners in London (two sites), Basel, Munich and Melbourne. The objective was to identify the most robust neuroanatomical abnormalities in subjects at UHR, and to compare UHR subjects who subsequently made a transition to psychosis with UHR subjects who did not. At each site, subjects were scanned at first clinical presentation, and followed clinically for an average of two years, so that they could then be subcategorized according to clinical outcome. The MRI data from each site were combined to form a large UHR sample, which was subdivided into subjects who had developed psychosis and subjects who had not. MRI data from a number of matched healthy controls were also acquired at each site.

The first prediction, based on data from previous studies (Phillips, Velakoulis et al. 2002, Job, Whalley et al. 2003, Pantelis, Yucel et al. 2003, Garner, Pariante et al. 2005, Job, Whalley et al. 2005, Velakoulis, Wood et al. 2006, Borgwardt, McGuire et al. 2007, Borgwardt, Riecher-Rossler et al. 2007, Borgwardt, McGuire et al. 2008, Fornito, Yung et al. 2008, Koutsouleris, Schmitt et al. 2009, Witthaus, Kaufmann et al. 2009, Buehlmann, Berger et al. 2010), was that the UHR group as a whole would show regional volumetric differences relative to controls that were qualitatively similar to those seen In patients with schizophrenia. The main hypothesis was that UHR subjects who later developed

psychosis would show differences in grey matter volume relative to those who did not in the inferior frontal, parahippocampal, and superior temporal cortex, areas most frequently implicated in previous studies. Critically, these predictions were based on the results of previous single centre studies on people at risk for psychosis or with a first episode of psychosis (Job, Whalley et al. 2003, Pantelis, Velakoulis et al. 2003, Farrow, Whitford et al. 2005, Garner, Pariante et al. 2005, Borgwardt, McGuire et al. 2008, Fornito, Yung et al. 2008, Sun, Phillips et al. 2009, Takahashi, Wood et al. 2009, Takahashi, Wood et al. 2009).

3.2. Methods

3.2.1. Sample

All the UHR subjects were recruited from specialised clinical services for people at high risk of psychosis in London, Basel, Melbourne and Munich. In total there were data from 182 patients at UHR. Most (168/182, 93%) of the UHR group had never taken antipsychotics or mood stabilizers; 14 (7%) had been exposed to antipsychotics, the mean exposure time was 13 months (SD=19.3). At each site, healthy controls from the same geographical area as the UHR subjects were recruited through local advertisement. The total control sample comprised 167 subjects, and was comparable to the total UHR group with respect to gender, age, and ethnicity (details are reported in **Table 3.2**.). Recruitment details are reported in Chapter 2, sections 2.2. and 2.2.2.

In the 30.6 months (mean follow up period; SD=10.4) subsequent to scanning, 48 (26.4%) of the UHR sample developed psychosis (UHR-T), and 134 did not (UHR-NT). Each site yielded a dataset that included an UHR-T group, an UHR-NT group and a control group, with the exception of one of the London datasets, from which the UHR-T group too small (n=2) to be included in the combined UHR-T versus UHR-NT

comparison (**Table 3.1**.). Clinical measures for each site are reported in Appendix 2.

Table 3.1. Number of subjects for site

Site	UHR-NT	UHR-T	Healthy Controls
London (Institute of Psychiatry)	19 (90.5%)	2 (9.5%)	27
London (Maudsley Hospital)	42 (84%)	8 (16%)	37
Basel	23 (65.7%)	12 (34.3%)	22
Munich	24 (60%)	16 (40%)	42
Melbourne	26 (72.2%)	10 (27%)	39
Total	134 (73.6%)	48 (26.4%)	167

3.2.2. Image acquisition

At all five sites, volumetric MR images were acquired using a T1-weighted protocol. At four sites the scanner field strength was 1.5T, at one it was 3T. Three sites used General Electric scanners, and two used Siemens scanners. The details of the image acquisition sequence varied between scanners, as reported in Chapter 2, section 2.3.2.

3.2.3. Data analysis

3.2.3.1. Sociodemographic data and clinical parameters

Differences in demographics and clinical profile between groups were examined using one-way analysis of variance (ANOVA) for parametric data, and a chi square test for non-parametric data, with the Statistical Package for the Social Sciences 17.0 (SPSS 17.0 for Windows), (**Table 3.2.**). Sociodemographic characteristics of the study samples by site are reported in Appendix 3 (Table A3.1.).

3.2.3.2. Preprocessing

Group-related differences in gray matter volume were examined using Voxel-based Morphometry (VBM), as implemented in SPM8 software (http://www.fil.ion.ucl.ac.uk/spm) running under Matlab 7.1 (Math

Works, Natick, MA, USA). First, T1-weighted 1 volumetric images were pre-processed using the Diffeomorphic Anatomical Registration using Exponentiated Lie algebra (DARTEL)(Ashburner 2007) SPM8 toolbox. This approach involves the creation of a study-specific template and the segmentation of each individual image using such template, with the aim of maximizing accuracy and sensitivity (Yassa and Stark 2009). The following steps were followed for VBM preprocessing: (1) checking for scanner artifacts and gross anatomical abnormalities for each subject; (2) setting the image origin to the anterior commissure; (3) using the DARTEL toolbox to produce a high-dimensional normalization protocol (Ashburner 2007); (4) checking for homogeneity across the sample; and (5) using standard smoothing (i.e., 8 mm). A "modulation step" was also included in the normalization in order to preserve the information about the absolute gray matter values (Ashburner and Friston 2000, Mechelli, Price et al. 2005). After this preprocessing, smoothed, modulated, normalised data were obtained and were subsequently used for the statistical analysis.

3.2.3.3. Statistical analyses

Two statistical analyses were performed using SPM8 software. First, an analysis of variance (ANOVA) was used to compare grey matter images from UHR subjects (UHR-T and UHR-NT combined) and healthy controls. In this analysis, scanner site was modelled as a factor, resulting in a total of 10 experimental groups. Second, an analysis of variance (ANOVA) was performed to compare grey matter images from UHR subjects who later became psychotic (UHR-T), UHR subjects who did not become psychotic (UHR-NT), and healthy controls. In this analysis, scanner site was again modelled as a factor, resulting in a total of 14 experimental groups (one UHR-NT group was too small for analysis; see above). Including scanner site as a factor in the statistical analysis allowed to (i) model scanner-related variance in the data which had the impact of reducing error variance and increasing statistical sensitivity and (ii) examine the impact of scanner site by testing for

scanner effects and scanner x group interactions. To assess how much of the inter-individual variance in regions which differed between UHR-T and UHR-NT was explained by diagnostic group and scanner site respectively, the η_p^2 measure of effect size was performed in SPSS. In both analyses, age, gender, ethnicity and use of medication were modelled as covariates of no interest to reduce the potential impact of these variables on the findings. In order to identify regionally specific changes that were not confounded by global differences, the proportional scaling option was used. Statistical inferences were made at $p < 0.05$ after family-wise error (FWE), with an extent threshold of 5 voxels.

3.2.3.4. ROI analyses

In addition to the whole-brain analysis, regions of interest (ROIs) were used to examine between group differences in areas where volumetric abnormalities have previously been identified in studies of people at high risk for psychosis, or patients with first episode psychosis (Job, Whalley et al. 2003, Farrow, Whitford et al. 2005, Job, Whalley et al. 2005). These comprised the left parahippocampal gyrus (-23, 6, -20), (Farrow, Whitford et al. 2005), right inferior frontal gyrus (45, 37, 0), (Job, Whalley et al. 2003), and the left superior temporal gyrus (-49, -31, 2), (Job, Whalley et al. 2005). The coordinates from the previous studies that had reported the most significant effects in these regions were included in a mask; importantly, none of these previous studies had included data from subjects who participated in the present investigation. Using the simpleROIbuilder toolbox (http://www.fil.ion.ucl.ac.uk/spm/ext/), I created a mask which included the three chosen regions of interest. This mask consisted of three spheres with a radius of 8 mm corresponding to the three regions of interest and comprising a total of 758.71 voxels. Within the mask, statistical inferences were made at $p < 0.05$ after FWE correction for multiple comparisons.

3.3. Results

3.3.1. Sociodemographic data and clinical parameters

There were no significant differences between the UHR-T, UHR-NT and healthy control groups in age, gender, total grey matter volume and ethnicity (**Table 3.2.**). Clinical characteristics per site are reported in Appendix 2.

Table 3.2. Sociodemographic data and global brain volumes of study samples

Characteristics	**Healthy Controls** (n= 167)	**UHR-T** (n= 48)	**UHR-NT** (n= 134)	**Significance**
Age (mean, SD)	23.5 ± 4.2	22.73 ± 4.5	23.3 ± 5.3	$F_{(2, 346)}$ = 0.552 p = 0.58
Gender (Female/Male)	63/104	14/34	51/83	χ^2_2 = 2.541 p = 0.28
Ethnicity Caucasian Black Asian Mixed	 140/167 11/167 10/167 6/167	 42/48 1/48 0/48 5/48	 106/134 7/134 6/134 15/134	χ^2_6 = 11.122 p = 0.09
Handedness Right Left Ambidextrous	 147 14 6	 44 3 1	 126 6 2	χ^2_4 = 3.355 p = 0.50
GMV (mean, SD)	0.948 ± 0.11	0.945 ± 0.08	0.937 ± 0.10	$F_{(2, 346)}$ = 0.450 p = 0.64

3.3.2. Differences between the UHR and control groups

The UHR group had less grey matter volume than controls (at $p < 0.05$ after FWE correction) in three areas of frontal cortex: the medial orbital gyrus and the gyrus rectus bilaterally, and the right anterior cingulate gyrus (**Figure 3.1.**, **Table 3.3.**). In these regions there was no significant effect of medication even at trend level ($p < 0.05$ uncorrected). There were no areas where UHR subjects had more grey matter volume than controls.

Figure 3.1.

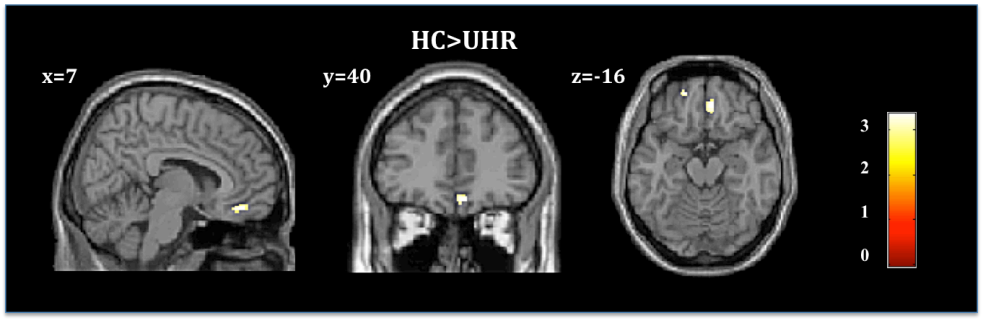

Figure 3.1. Differences between the ultra UHR and HC groups. The images show the medial orbital region, where the total UHR sample showed reduced gray matter volume relative to HC ($p < 0.05$ FWE corrected).

Table 3.3. MNI coordinates

HC>UHR Area	Hem.	x	y	z	Cluster size (No. of Voxels)	z Score	p Value
Medial orbital gyrus	Right	7	40.5	-16.5	130	5.44	0.001
	Left	-18	52.5	-16.5	30	4.94	0.005
Cingulate gyrus	Right	12	58.5	9	50	5.35	0.001
Gyrus rectus	Right	3	25.5	-25.5	73	4.94	0.01
	Left	-4	25.5	-22.5		4.80	0.02

Table 3.3. MNI coordinates and *z* scores for regions showing differences in gray matter volume between UHR and HC groups. Abbreviations: Hem, hemisphere; UHR, ultra high risk, HC, healthy controls.

3.3.3. Differences between UHR subjects who did and did not develop psychosis

Region-of-interest analysis revealed reduced grey matter volume in the UHR-T relative to the UHR-NT group in the anterior part of the left parahippocampal gyrus (bordering the uncus), ($p < 0.05$ after FWE correction, **Figure 3.2.**), where the difference between UHR-T and UHR-NT accounted for 14% of the total variance. In this region, there was no significant effect of medication even at trend level ($p < 0.05$ uncorrected). Plotting of gray matter values revealed that this reduction was evident in each of the four sites examined for this contrast (**Figure**

3.2.). There were no significant differences in the other ROIs (i.e. in the right inferior frontal gyrus, and the left superior temporal gyrus).

In order to examine the predictive value of gray matter volume in the left parahippocampal gyrus, I extracted gray matter values from the peak voxel and performed a series of cross-validation analyses using a predictive linear model in SPSS software. This involved developing a predictive model based on a data set from a single scanner site and testing it in each of the other data sets; the average predictive accuracy was 62% (sensitivity = 61%; specificity = 65%). I also performed a three-fold cross-validation analysis irrespective of scanner site which involved developing a predictive model based on two thirds of the total sample and testing it in the remaining one third; this yielded an average accuracy of 67% (sensitivity = 68%; specificity = 66%).

Figure 3.2.

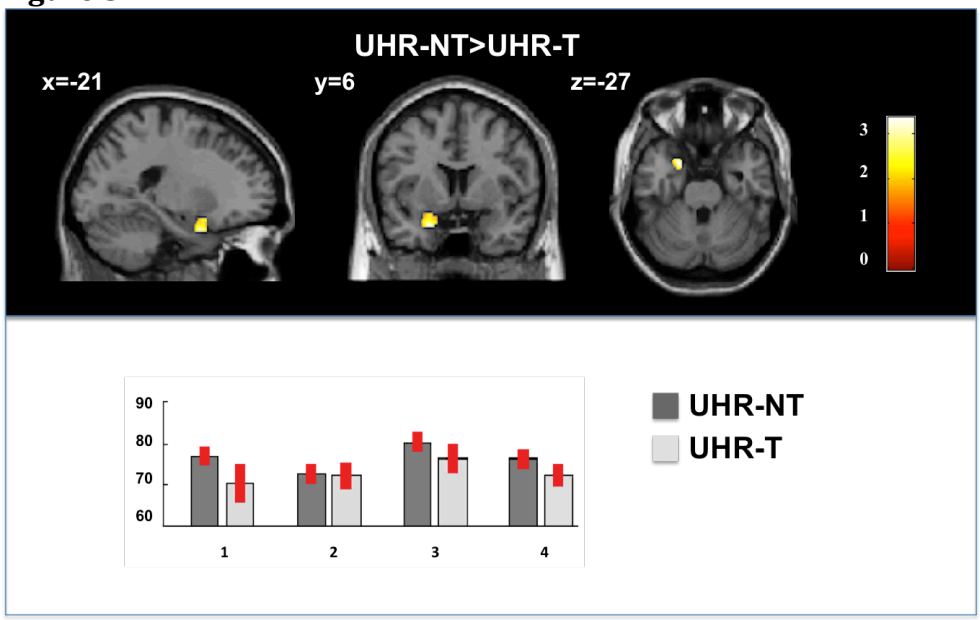

Figure 3.2. Differences between UHR-T and UHR-NT. The UHR-T individuals had less gray matter volume than did the UHR-NT individuals in the left parahippocampal gyrus, bordering the uncus (MNI [Montreal Neurological Institute] coordinates x, y, and z: −21, 6, and −27, respectively). For visualization purposes, effects are displayed at $p < 0.05$ uncorrected. The plot shows mean gray matter volumes for the two UHR subgroups at each site (x-axis: 1 indicates London, United Kingdom; 2, Basel, Switzerland; 3, Munich, Germany; and 4, Melbourne, Australia); values on the y-axis refer to cubic millimeters per voxel. Error bars represent SD.

3.4. Discussion

MRI was used to study a large sample of UHR subjects created by pooling data from five sites. The UHR subjects were followed clinically subsequent to scanning and subcategorised according to which individuals developed psychosis and which did not.

On the basis of previous MRI studies of smaller UHR samples collected at single sites, (Phillips, Velakoulis et al. 2002, Job, Whalley et al. 2003, Pantelis, Yucel et al. 2003, Garner, Pariante et al. 2005, Job, Whalley et al. 2005, Velakoulis, Wood et al. 2006, Borgwardt, McGuire et al. 2007, Borgwardt, Riecher-Rossler et al. 2007, Borgwardt, McGuire et al. 2008, Fornito, Yung et al. 2008, Koutsouleris, Schmitt et al. 2009, Witthaus, Kaufmann et al. 2009, Buehlmann, Berger et al. 2010) I first tested the hypothesis that the UHR group as a whole would show volumetric abnormalities relative to controls that were qualitatively similar to those seen in patients with schizophrenia. Consistent with this prediction, the UHR group expressed significant reductions in grey matter volume in the prefrontal and anterior cingulate cortex ($p < 0.05$ after FWE correction), areas that have been consistently implicated in volumetric neuroimaging studies of schizophrenia (Steen, Mull et al. 2006). Interestingly, reductions in similar regions of the prefrontal cortex have been associated with high scores in psychometric schizotypy in healthy individuals, further providing support for a phenomenological and phonotypical continuum with schizophrenia spectrum disorders (Ettinger, Williams et al. 2012). In contrast, I did not identify areas where there was more grey matter volume in the UHR sample than in controls.

The main prediction was that the UHR subjects who later developed psychosis would show differences in regional grey matter volume in the inferior frontal, parahippocampal, and superior temporal cortex compared to those who did not become psychotic. This hypothesis was in part confirmed: the subgroup that subsequently became psychotic

showed relatively reduced grey matter volume in the anterior part of the left parahippocampal gyrus, bordering the hippocampal uncus (p < 0.05 after FWE correction). In this area, there was less grey matter in UHR-T than controls (p < 0.05 corrected), but no significant difference between the UHR-NT and control groups. A series of cross-validation analyses also revealed that gray matter volume in this region allowed discrimination between UHR-T and UHR-NT with an accuracy of up to 67%. In contrast to some previous single centre studies, (Job, Whalley et al. 2003, Job, Whalley et al. 2005) no significant differences were found in either the inferior frontal or superior temporal gyri.

Reductions in parahippocampal volume have been reported in high risk subjects relative to controls (Job, Whalley et al. 2003), as have alterations in parahippocampal function (Allen, Stephan et al. 2010). Over time reductions in parahippocampal volume have been described in high risk subjects with transient or isolated psychotic symptoms (Job, Whalley et al. 2005) and longitudinal reductions have been described in high risk subjects who developed psychosis (Buehlmann, Berger et al. 2010). Moreover cross-sectional comparisons indicate that patients with first episode psychosis have thinner parahippocampal cortical thickness than both controls and UHR subjects. Past studies suggest that the parahippocampal region is one of the most robust sites of volume reduction in chronic schizophrenia (Shenton, Kikinis et al. 1992, Shenton, Dickey et al. 2001, Seidman, Pantelis et al. 2003, Steen, Mull et al. 2006). Contemporary animal models of psychosis propose that altered parahippocampal activity drives subcortical dopamine dysfunction (Grace 2012). These observations are consistent with the notion that the parahippocampal cortex is critically implicated in psychotic disorders.

At the time of scanning, the UHR-T and UHR-NT groups were clinically indistinguishable; the volumetric differences that were observed between them could be interpreted as neurobiological markers of an

especially increased vulnerability to psychosis, or early manifestations of a neuropathological process underlying the transition to psychosis. Because the data in the present work were collected at a single cross-sectional time-point, it cannot be determined at what stage these differences first emerged. This issue could be addressed by longitudinal neuroimaging studies of subjects at different time-points within the prodromal phase of psychosis.

A limitation of the present work is that that the data were collected on different scanners and using different acquisition sequences (Meda, Giuliani et al. 2008, Segall, Turner et al. 2009, Stonnington, Chu et al. 2010). Nevertheless the present results are unlikely to represent an artefact due to the use for different scanners for several reasons. Firstly, I sought to control for these effects by only using MRI data collected with T1-weighted sequences and by modelling scanner site as an independent factor in the statistical analysis. Secondly, a comparable proportion of controls, non-converters and converters were scanned at each scanning site, with the exception of one of the London datasets which was therefore excluded from the UHR-T versus UHR-NT comparison. Thirdly, plotting of gray matter values suggested that the differences between UHR-T and UHR-NT groups were evident not only across different centres but also within each site and therefore cannot be explained by inter-scanner differences (**Figure 3.2**). Fourthly, when the impact of scanner site was examined, no evidence of either scanner effects or scanner x group interactions was found in regions which differed between groups, even when lowering the statistical threshold to $p < 0.05$ (uncorrected). Finally, the present approach to the integration of multi-scanner data within the same statistical model has been employed successfully in previous studies which also combined different scanners and acquisition sequences (Meda, Giuliani et al. 2008, Stonnington, Tan et al. 2008, Segall, Turner et al. 2009, Suckling, Barnes et al. 2010); these studies typically found that scanner differences were substantially less than group differences. Consistent

with this, in the present work scanner-related variance (11%) was less than group-related variance (14%) in the left parahippocampal region which differed between UHR-T and UHR-NT groups. The impact of the use of different scanners and acquisition sequences could be further controlled for by scanning the same individuals at each site, but this was not feasible in the present investigation.

The data from the present study suggest that in future it may be possible to use MRI to facilitate the prediction of psychosis in those at high risk (Job, Whalley et al. 2006). This would be particularly useful in the clinical management of subjects at UHR, as it is difficult to predict which individuals will go on to develop psychosis on the basis of their clinical features (Yung, Phillips et al. 2004). As a result, it is not possible to focus the delivery of clinical resources, such as putatively preventative treatments (McGorry and Killackey 2002), to the subgroup of UHR subjects who will later become psychotic. Clinical application of neuroimaging in this context requires the identification of predictive markers at an individual level, whereas the present data represent group differences. Image analysis methods that classify individual subjects according to patterns of data associated with diagnostic categories may provide a means of addressing this issue (Mourao-Miranda, Bokde et al. 2005).

3.4.1. Conclusions

In conclusion, the UHR is associated with alterations in regional grey matter volume, and within this population, reductions in the parahippocampal region may be specifically linked to the later onset of psychosis. These findings suggest that neuroimaging data may facilitate the prediction of illness in subjects at high risk of psychosis, and inform the development of new interventions designed to delay or prevent its onset.

4. Reduced parahippocampal cortical thickness in subjects at ultra high risk for psychosis: a multi-centre voxel-based cortical thickness study

(Adapted from published paper, Appendix 1, A1.2.)

This study was published in Psychological Medicine under the title "Reduced parahippocampal cortical thickness in subjects at ultra-high risk for psychosis"

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4.1. Introduction

As discussed in Chapter 1 and Chapter 3, the vast majority of structural neuroimaging studies in UHR individuals have focused on gray matter

volume, however, neuroanatomical alterations in psychosis may be expressed not only in terms of gray matter volume but also as subtle changes in cortical thickness. While the analysis of gray matter volume returns a mixed measure that depends on local cortical thickness as well as cortical folding and gyrification (i.e. cortical surface area), the analysis of cortical thickness is considered to specifically target the presence of cortical atrophy (Hutton, De Vita et al. 2008, Hutton, Draganski et al. 2009). Therefore the two approaches provide complementary information and one can be more sensitive than the other depending on the process underlying neuroanatomical changes. Cortical thickness in the human brain can be examined using the automated data analysis of T1-weighted images (Hutton, De Vita et al. 2008). Application of this approach in patients with first episode and established schizophrenia suggests that there is a cortical thinning in the anterior cingulate (Narr, Bilder et al. 2005, Fornito, Yucel et al. 2008, Schultz, Koch et al. 2010), prefrontal (Narr, Bilder et al. 2005, Venkatasubramanian, Jayakumar et al. 2008, Schultz, Koch et al. 2010), temporal (Narr, Bilder et al. 2005, Fornito, Yucel et al. 2008), and occipital (Narr, Toga et al. 2005) cortices. In UHR subjects, reduced cortical thickness has been reported in the prefrontal, anterior cingulate, inferior parietal, superior temporal and parahippocampal cortices compared to healthy controls (Jung, Kim et al. 2011). One study has reported that in UHR individuals who subsequently developed psychosis the anterior cingulate cortex was thinner than in UHR that did not become ill (Fornito, Yung et al. 2008). A longitudinal study has reported a progressive thinning in the anterior cingulate, precuneus and temporo-parietal-occipital cortex compared to UHR-NT and healthy controls (Ziermans, Schothorst et al. 2012). Another study did not find differences in cortical thickness between individuals at UHR, patients with a first psychotic episode and healthy controls, but the groups differed in cortical thickness asymmetry (Haller, Borgwardt et al. 2009).

As with volumetric studies, the findings from studies of cortical thickness in UHR subjects may have been inconclusive due to the relatively small sample sizes examined to date. A multi-centre approach was therefore adopted in the present work. The main aim was to combine MRI data from multiple centres and measure cortical thickness in large samples of UHR subjects and healthy controls. The second aim was to compare cortical thickness in UHR subjects who subsequently did or did not developed psychosis. Whole-brain magnetic resonance images were acquired from individuals at UHR for psychosis and healthy controls at four psychiatric research centres in London, Basel, Munich and Melbourne. At each site, subjects were scanned at first clinical presentation, and followed up clinically or in the context of research projects for at least two years, so that they could be subcategorized according to psychosis outcome. The MRI data from each site were combined to form a large UHR sample, which was subdivided into subjects who later had developed psychosis and subjects who had not. The thickness of the cerebral cortex was assessed using a voxel-based cortical thickness (VBCT) approach that generates maps from MRI data in which each voxel in the gray matter is assigned a thickness value and regionally specific differences are compared on a voxel-by-voxel basis (Hutton, De Vita et al. 2008, Hutton, Draganski et al. 2009). This method differs from others reported in the literature as it does not require the construction of a three-dimensional model for extracting cortical thickness values. For instance, surface-based techniques involve the generation of surface models that are driven by image information and surface geometry to fit the gray and white matter surfaces of the image (Fischl and Dale 2000, Hutton, De Vita et al. 2008). Cortical thickness is subsequently defined at surface points and is computed based on the measure of the distance between them. Another approach involves extracting only the surface between the gray and the white matter and then mapping towards the surface the thickness values that are derived by calculating the distance between voxels in the cortex and the surface (Lerch and Evans 2005, Hutton, De

Vita et al. 2008). In contrast, using the VBCT technique, gray and white matter boundaries are defined on the basis of whole voxel information (Jones, Buchbinder et al. 2000, Hutton, De Vita et al. 2008) and cortical thickness is calculated at every volumetric point within the cortex and based on the length of the trajectory from one boundary to another (Hutton, De Vita et al. 2008).

The first prediction, based on data from previous studies (Narr, Bilder et al. 2005, Fornito, Yucel et al. 2008, Fornito, Yung et al. 2008, Schultz, Koch et al. 2010, Jung, Kim et al. 2011, Ziermans, Schothorst et al. 2012) was that the UHR group as a whole would show differences in cortical thickness relative to controls in areas that have previously been identified in studies of cortical thickness in UHR subjects and patients with first episode psychosis: the frontal, anterior cingulate, parahippocampal, temporal, parietal cortices and the precuneus. The second prediction was that within the UHR sample subjects who developed psychosis subsequent to scanning would show more pronounced cortical thickness abnormalities in these regions (Narr, Bilder et al. 2005, Fornito, Yucel et al. 2008, Fornito, Yung et al. 2008, Schultz, Koch et al. 2010, Jung, Kim et al. 2011, Ziermans, Schothorst et al. 2012) than those who did not.

4.2. Methods

4.2.1. Sample

All the UHR subjects were recruited from specialised clinical services for this group in London (i.e. 1.5T scanner), Basel, Melbourne and Munich. Data were combined from these four sites. This sample partially overlaps with the multi-centre sample described in Chapter 3 (Mechelli, Riecher-Rossler et al. 2011), and includes subjects that participated in previous single centre studies of grey matter volume in this population. In total there were MRI data from 167 UHR subjects. MRI data acquired from healthy volunteers from the same geographical

area as the UHR subjects at each site were combined to form a control dataset. The total control sample comprised 150 subjects, and was comparable to the total UHR sample with respect to gender, age, and ethnicity (details are reported in **Table 4.1 and 4.2.**). Recruitment details are reported in Chapter 2, sections 2.2. and 2.2.2.

Table 4.1. Number of subjects for site

Site	**UHR-NT**	**UHR-T**	**Healthy Controls**
London Maudsley Hospital	44 (78.6%)	12 (21.4%)	47
Basel	23 (65.7%)	12 (34.3%)	22
Munich	24 (60%)	16 (40%)	42
Melbourne	26 (72.2%)	10 (27.8%)	39
Total	117 (70%)	50 (30%)	150

In the following 30.6 months (SD=10.4), 50 (30%) of the UHR individuals developed psychosis (UHR-T) and 117 did not (UHR-NT). Transition to psychosis during the follow-up period was established according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria based on clinical consensus between at least two experienced psychiatrists. Most of the UHR group (147/167, 84%) had never taken antipsychotics at time of scanning; 20 (12%) had been exposed to antipsychotics; the mean antipsychotic medication exposure time was 7.5 weeks (SD=11.1). UHR participants that were receiving medication were scanned at the London or Basel sites. Clinical characteristics of the UHR subjects are reported in Appendix 2.

4.2.2. Image acquisition

At all four sites, volumetric MR images were acquired using scanner field strengths of 1.5T and a T1-weighted protocol. Two sites used General Electric scanners (i.e. London and Melbourne), and two used Siemens scanners (i.e. Basel and Munich). The details of the image acquisition sequence are reported in Chapter 2, section 2.3.2.

4.2.3. Data analysis

4.2.3.1. Sociodemographic data

Sociodemographic differences between groups were examined using one-way analysis of variance (ANOVA) for parametric data, and a chi square test for non-parametric data, as implemented in the Statistical Package for the Social Sciences 19.0 (SPSS 19.0 for Windows), (**Table 4.2.**). Sociodemographic characteristics of the study samples by site are reported in Appendix 3 (Table A3.2.).

4.2.3.2. Preprocessing

Preprocessing for the analysis of cortical thickness was carried out using the procedure described by Hutton and colleagues (Hutton, De Vita et al. 2008, Hutton, Draganski et al. 2009). In brief, all the images were visually checked and resampled to a voxel size of 1 mm^3 using trilinear interpolation. Using the unified segmentation procedure implemented in SPM8 (http://www.fil.ion.ucl.ac.uk/spm), the images were segmented into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) (Ashburner 2007). For each subject, this resulted in a set of 3 images in the same space as the original T1-weighted image, in which each voxel was assigned a probability of being GM, WM, and CSF, respectively. A voxel-based cortical thickness (VBCT) map was created for each subject using the GM, WM, and CSF segments created in the previous step (Hutton, De Vita et al. 2008). This method is implemented as a toolbox for SPM (Hutton, De Vita et al. 2008, Hutton, Draganski et al. 2009). In brief, it uses the input tissue probability maps and a transformed labeled brain atlas (http://www.fil.ion.ucl.ac.uk/spm/ext/#IBASPM). Starting from the initial estimate of the GM/WM boundary, layers of 1 voxel in thickness are successively added to surround the WM allowing voxels to be identified where the GM from different sides of a sulcus was in contact. Once all GM voxels have been processed in this way, Laplace's equation is solved for all voxels between the final GM/WM and GM/CSF boundaries resulting in a scalar field that makes a smooth transition

from 1 boundary to the other. The gradient of this field at each point forms a unique trajectory connecting the two boundaries, and the thickness at each point is calculated by integrating along these trajectories. The resulting VBCT maps are created in the space of the original input images which contain cortical thickness values within voxels identified as cortical GM and zeros outside the cortex. DARTEL (Ashburner 2007), an algorithm for diffeomorphic image registration which is implemented as a toolbox for SPM8, was used to warp the VBCT maps into a new group-specific reference space representing an average of all the subjects. This procedure uses the GM and WM segments estimated from the original T1-weighted images to calculate a group-specific template and the deformation fields required to warp data from each subject to the new template. Each VBCT map was warped to the new template using the corresponding subject specific deformation field and was resampled to an isotropic voxel size of 1.5 mm^3 using trilinear interpolation. The warped VBCT maps were scaled by the Jacobian determinant of the deformations to account for stretching and compression and subsequently smoothed with a 6-mm Gaussian kernel then divided by a binary mask of each original VBCT map which had been identically warped, scaled, and smoothed. A Gaussian kernel of 6-mm was chosen because, when investigating cortical thickness, it is important to keep smoothing to a minimum so that any abrupt changes in thickness that may occur at the boundaries between cortical areas are not obscured (Hutton, De Vita et al. 2008). This procedure results in smoothed warped VBCT maps for which the Gaussian smoothing kernel applied in the warped space has been projected into the native space of the subject, and the cortical thickness values are preserved over a region the size of the smoothing kernel. Before performing the statistical analysis on the smoothed warped VBCT maps, I checked for homogeneity across the sample.

4.2.4. Statistical analyses

The statistical analysis was performed using SPM8 software on the smoothed warped VBCT maps. An analysis of variance was used to compare cortical thickness in UHR-T, UHR-NT and healthy controls. Scanner site was modeled as an additional factor, resulting in a total of 12 experimental groups. Including scanner site as a factor in the statistical analysis allowed us to model scanner-related variance in the data, which had the effect of reducing error variance and increasing statistical sensitivity. Age, gender, ethnicity and handedness were also modelled as covariates of no interest to minimize any confounding effect of these variables on the findings. A region of interest (ROI) approach was used to examine between group differences in areas where abnormalities in cortical thickness have been identified in MRI studies of individuals at UHR or with a first episode of psychosis, excluding studies that included data from subjects who participated in the present investigation. These comprised the parahippocampal gyrus, inferior frontal gyrus, anterior cingulate, superior temporal gyrus, inferior parietal gyrus, and the precuneus (Narr, Bilder et al. 2005, Schultz, Koch et al. 2010, Jung, Kim et al. 2011, Ziermans, Schothorst et al. 2012). Using WFU PickAtlas (http://www.nitrc.org/projects/wfu_pickatlas/) a mask was created that included the six chosen regions of interest and comprised a total of 11,700 voxels. Within this mask, statistical inferences were made using a statistical threshold of $p < 0.05$ after familywise error (FWE) correction for multiple comparisons as calculated in SPM8. Trends that did not survive correction for multiple comparisons ($p < 0.001$ uncorrected) are reported but not discussed. For completeness I also performed a whole-brain analysis using a statistical threshold of $p < 0.05$ after FWE correction for multiple comparisons.

4.3. Results

4.3.1.Sociodemographic data

No statistically significant differences were noted among the UHR-T, UHR-NT, and control groups in age, sex, ethnicity and handedness (**Table 4.2.**).

Table 4.2. Sociodemographic characteristics of the study samples

Characteristics	**Healthy Controls** (n= 150)	**UHR-T** (n= 50)	**UHR-NT** (n= 117)	**Significance**
Age (mean, SD)	23.4 ± 4.3	22.9 ± 4.6	23.3 ± 5.3	$F_{(2, 315)}$ = 0.221 p = 0.801
Gender (Female/Male)	51/99	36/14	83/51	χ^2_2 = 2.927 p = 0.231
Ethnicity Caucasian Black Asian Mixed	 128/150 7/150 9/150 6/150	 43/50 1/50 0/50 6/50	 96/117 5/117 5/117 11/117	 χ^2_6 = 8.320 p = 0.216
Handedness Right Left Ambidextrous	 132/150 12/150 6/150	 45/50 4/50 1/50	 111/117 4/117 2/117	 χ^2_4 = 4.164 p = 0.384
Medication (number of subjects receiving antipsychotics)	n/a	5/50	21/117	χ^2_1 = 1.684 p = 0.194

4.3.2. Differences between the UHR and control groups

Within the regions of interest, the cortex was thinner in the UHR group than in controls in the right parahippocampal gyrus (p < 0.05 FWE corrected; z-score= 4.06; MNI coordinates x= 25, y= -4, z= -13 see **Figure 4.1**). There was also a trend (p < 0.001 uncorrected) for a thinner cortex in the UHR group compared to controls in the inferior part of the left parahippocampal gyrus (z-score =3.42: MNI coordinates

x= -20, y= -7 z= -30). Plotting of the cortical thickness values revealed that the reduction in right parahippocampal cortical thickness was evident in the data from each of the four sites (**Figure 4.1**). In contrast, there were no areas in which UHR individuals had thicker cortex than healthy controls. The whole-brain analysis did not identify significant differences in cortical thickness between the UHR group and healthy controls at $p < 0.05$ (FWE corrected).

Figure 4.1.

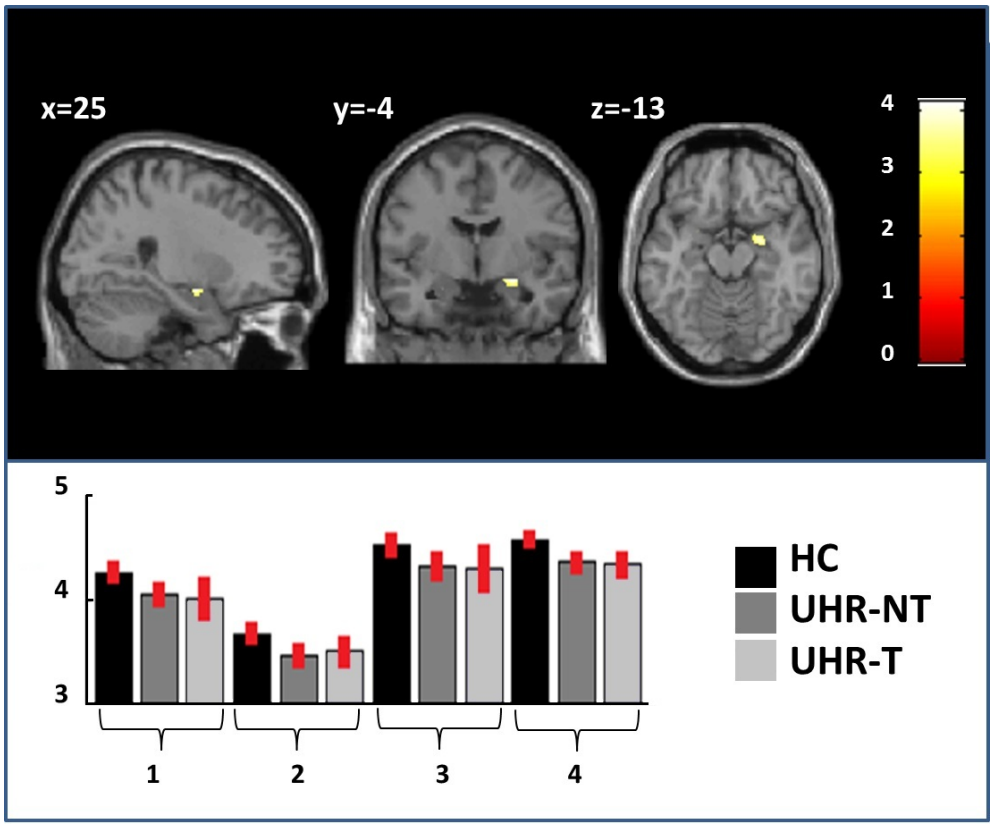

Figure 4.1. Cortical thickness differences between the UHR and HC groups. Right parahippocampal region where the total UHR sample showed cortical thinning relative to healthy controls (HC); ($p < 0.05$ after FWE correction). For visualization purposes, effects are displayed at $p < 0.001$ uncorrected. The plot shows cortical thickness values for the healthy control group and two UHR subgroups at each site (x axis: 1= London, 2=Basel, 3=Melbourne, 4=Munich); values on the y axis refer to millimeters (mm). Error bars represent SD.

4.3.3. Differences between UHR subjects who did and did not develop psychosis

No differences were observed for the comparison between UHR-T and UHR-NT at $p < 0.05$ after FWE correction. At a less conservative statistical threshold ($p < 0.001$ uncorrected), there was a trend for cortical thinning in the UHR-T group in the orbital part of the left inferior frontal gyrus (z-score=3.32; MNI coordinates x= -33, y= 32, z= -15). The whole-brain analysis revealed no significant differences between the UHR-T and UHR-NT groups at $p < 0.05$ (FWE corrected).

4.4. Discussion

Previous neuroimaging studies have reported cortical thinning in schizophrenia, as well as in first episode psychosis and in UHR subjects (Narr, Bilder et al. 2005, Fornito, Yucel et al. 2008, Fornito, Yung et al. 2008, Schultz, Koch et al. 2010, Jung, Kim et al. 2011, Ziermans, Schothorst et al. 2012). However, the results of these studies have not always been consistent; this may reflect the recruitment of relatively small samples, the use of different study designs, and the investigation of samples that were heterogeneous with respect to age, duration of illness and exposure to treatment. In the present doctoral work, the impact of these potential methodological pitfalls was reduced by assessing cortical thickness in a relatively large sample of individuals at UHR for psychosis, all of whom were scanned at a similar stage of illness, when they first presented with clinical high risk symptoms. Most of the sample had not been treated before. The first hypothesis was that the UHR group as a whole would show differences in regional cortical thickness relative to controls, and that these would be most evident in areas that have previously been identified as sites of cortical thickness or volume abnormalities in studies of UHR and first episode subjects (i.e. the temporal, frontal, parietal, anterior cingulate, parahippocampal cortices and the precuneus). This hypothesis was in part confirmed, in that it was found that the right parahippocampal cortex was thinner in the UHR group than in controls. This finding is

consistent with those of a recent investigation which also found reductions in thickness in other cortical areas (Jung, Kim et al. 2011). This result is also in line with evidence that the density of the parahippocampal gyrus is altered in UHR and familial high risk subjects (Job, Whalley et al. 2003). Furthermore, in first episode schizophrenia, altered right parahippocampal-lingual cortical folding and reduced cortical thickness have been found (Schultz, Koch et al. 2010). Finally, this region has also been identified as a site of functional (Allen, Seal et al. 2011) alterations in UHR subjects and is one of the most robust site of volume reduction (Seidman, Pantelis et al. 2003) and neuropathological abnormalities (Shenton, Dickey et al. 2001) in schizophrenia. In the study described in Chapter 3, differences between the UHR sample and controls were described in the ventral prefrontal and anterior cingulate cortex, but not in the parahippocampal cortex (Mechelli, Riecher-Rossler et al. 2011). However, in that study the subgroup of UHR subjects that subsequently developed psychosis had smaller left parahippocampal volumes than the UHR subjects who did not make a transition to psychosis. This volumetric finding was in a similar part of the parahippocampal gyrus to the site of the difference in cortical thickness between all UHR subjects and controls in the present work, although in the opposite hemisphere. Differences in the location of alterations in cortical thickness and cortical volume may reflect the fact that abnormalities in these two measures are not necessarily manifestations of the same underlying neuroanatomical changes. This would be consistent with recent evidence that cortical thickness and surface area are genetically and phenotypically independent and that the local cortical volume is more closely related to surface area than to cortical thickness (Winkler, Kochunov et al. 2010). Therefore differences between the results of the present work and the previous VBM study may be partially explained by the fact that the cortical thickness method does not take surface information into account. The volumetric reduction of the left parahippocampal gyrus reported in the study described in Chapter 3 (Mechelli, Riecher-Rossler et al. 2011)

may denote a marker of progression consistent with the results of earlier investigations (Pantelis, Velakoulis et al. 2003, Job, Whalley et al. 2005), whereas the thinning of the right parahippocampal gyrus may represent either a vulnerability marker (evident not only in the UHR stage but also in the earlier asymptomatic stage) or a specific marker of the UHR stage (not evident in the earlier asymptomatic stage). These two alternative options could be investigated by acquiring longitudinal neuroimaging data from individuals at high genetic risk.

The second hypothesis was that UHR subjects who went on to develop psychosis would already at baseline show more pronounced cortical thickness abnormalities than UHR subjects who did not. No significant differences that survived correction for multiple comparisons were observed. Two previous studies have investigated cortical thickness in UHR subjects in relation to clinical outcome. One study, which restricted its analysis to the anterior cingulate cortex, reported cortical thinning in this region in the UHR-T group compared to the UHR-NT (Fornito, Yung et al. 2008). The anterior cingulate cortex was included as one of the regions of interest based on previous studies (Narr, Toga et al. 2005, Jung, Kim et al. 2011, Ziermans, Schothorst et al. 2012), however an effect in this regions was not detected; this may be in part explained by methodological differences in thickness measurements and in ROIs investigated. The second study used MRI to scan UHR subjects at two time-points and compared longitudinal changes in cortical thickness in UHR individuals who did and did not make a transition to psychosis. The UHR-T group showed longitudinal reductions in several regions including anterior cingulate, precuneus and temporo-parietal-occipital areas compared to controls, whereas the UHR-NT group did not (Ziermans, Schothorst et al. 2012). In contrast no differences in cortical thickness were reported between UHR-T and UHR-NT at baseline. The inconsistency between previous studies and the present investigation could be in part explained by the use of different techniques to measure cortical thickness. In particular, the

studies by Fornito and colleagues and Ziermans and colleagues used two different surface-based methods (Fischl and Dale 2000, Kim, Singh et al. 2005, Fornito, Yung et al. 2008, Ziermans, Schothorst et al. 2012) while in the present work, a voxel-based method was used to derived cortical thickness values (Hutton, De Vita et al. 2008). Another study reported no baseline differences in cortical thickness between UHR and first episode psychosis and healthy controls, although the analysis of cortical asymmetry revealed significant group differences (Haller, Borgwardt et al. 2009). The lack of significant differences between UHR-T and UHR-NT may seem surprising, given that the present sample was relatively large, and abnormalities in functional and in other structural measures between these subgroups have previously been identified (Pantelis, Velakoulis et al. 2003, Borgwardt, McGuire et al. 2008, Mechelli, Riecher-Rossler et al. 2011, Allen, Luigjes et al. 2012). One possible explanation for the absence of significant differences in the present study is that there was some heterogeneity within the present UHR sample. Individuals can meet the UHR inclusion criteria through different patterns of clinical and cognitive symptoms, and it is unknown if these reflect distinct pathophysiological processes. Unfortunately, a stratification of the statistical analysis by type of UHR inclusion criteria was not possible in the present investigation as incorporating this additional factor would require much larger single centre sample sizes. The sample may also have been clinical heterogeneous with respect to the co-morbid anxiety, depression and substance abuse that are often present in UHR subjects (Svirskis, Korkeila et al. 2005).

Another possibility is that some subjects who met transition criteria subsequently have returned to lower levels of psychotic symptoms and higher levels of functioning, while some who did not meet criteria for transition actually have deteriorated in functioning over time. This latter group may be more likely to develop schizophrenia than the former group (Yung, Nelson et al. 2010). Thus the non-transitioned but

low functioning group may show brain imaging changes consistent with the ones observed in schizophrenia, but the high functioning transitioned cases may not.

The age range of the present sample of UHR individuals varied considerably across the four sites (age range 15-37, see Table A3.2., Appendix 3). Previous works indicates that the pattern of neuroanatomical findings in schizophrenia with a relatively early onset (e.g. before the age of 18) differs from that in schizophrenia with onset in adulthood and that the longitudinal trajectory of brain abnormalities varies with the age of onset (Gogtay, Vyas et al. 2011). Although age was modelled as a covariate of no interest in the statistical analysis, the fact that the UHR sample comprised individuals at different stages of brain development might have reduced the likelihood of detecting reliable differences.

A further potential contributory factor is that the MRI data were collected on different scanners, using different acquisition sequences. However, the present results are not likely to be significantly affected by the use of different scanners for several reasons. First, only MRI data collected with T1-weighted sequences were used and scanner site was modelled as an independent factor in the statistical analysis. Second, a comparable proportion of controls, UHR-NT and UHR-T were scanned at each scanning site. Third, plotting of gray matter values suggested that the differences between UHR and healthy control groups were evident also within each site and therefore cannot be explained by inter-scanner differences (**Figure 4.1**). Fourthly, when the impact of scanner site was examined, no evidence of scanner x group interactions was found in the region that differed between groups, even when lowering the statistical threshold to $p < 0.05$ (uncorrected). A scanner x group interaction was observed in the superior temporal gyrus (MNI coordinates x; y; z: -46; 18; -12 $p = 0.022$), which however did not significantly differ between HC and UHR or between UHR-T and UHR-

NT. Finally, the present approach to the integration of multi-scanner data has been employed successfully in previous studies that also combined different scanners and acquisition sequences (Stonnington, Tan et al. 2008, Segall, Turner et al. 2009, Suckling, Barnes et al. 2010). Nevertheless, it cannot be completely excluded an effect of scanner on the findings, and ideally multi-centre studies should employ the same acquisition sequence, and calibrate data across scanners using phantoms and common subjects to minimize the risk of scanner-related effects (Jack, Bernstein et al. 2008).

The whole-brain analysis did not identify significant differences in cortical thickness between the UHR group and healthy controls or between UHR-T and UHR-NT at $p < 0.05$ (FWE corrected). This could be in part explained by the heterogeneity of the UHR sample (e.g. symptoms, level of functioning, age), or by the use of different scanners and sequences. Nevertheless, when restricting the analysis to regions of interest, the method employed was able to detect a thickness reduction in the right parahippocampal gyrus in UHR group compared to the control group at a statistical threshold of $p < 0.05$ corrected (FWE). The reduction was evident across all the different scanning sites. Using the whole-brain approach did not enable to detect differences at a corrected level, this might indicate that group differences in the UHR population are distributed and diluted across the brain cortex.

4.4.1. Conclusions

In conclusion right parahippocampal thinning was observed in subjects at UHR for psychosis suggesting that thickness reduction in this region is related to the UHR symptomatology rather than the onset of psychosis. This cortical alteration could represent a marker of vulnerability to psychosis or, alternatively, a specific and distinctive marker of the UHR stage. No reliable differences in cortical thickness were found between subjects who did and did not go on to develop psychosis. Future multi-centre work in this area would benefit from the

subcharacterization of UHR individuals with different clinical and cognitive profiles, the recruitment of participants at the same stage of neurodevelopment and the use of a standardised image acquisition sequence.

5. Pattern classification analysis of structural data in a multi-centre sample of individuals at ultra high risk for psychosis

5.1. Introduction

In the last two decades, there has been growing interest in trying to identify those individuals in the prodromal stages of psychosis to provide immediate and targeted psychological and pharmacological interventions. To date, the identification of individuals at increased risk for psychosis relies mainly on clinical high risk criteria (Yung, Yuen et al. 2005, Schultze-Lutter 2007, Riecher-Rossler, Aston et al. 2008, Schultze-Lutter 2009, Schultze-Lutter, Klosterkotter et al. 2014). However, based on these sets of criteria, it is only possible to predict transition to psychosis in 18% of cases after 6 months of follow-up, 22% after 12 months, 29% after 24 months, and 36% after 3 years (Fusar-Poli, Bonoldi et al. 2012). Therefore additional biomarkers that can aid the identification of prodromal individuals are necessary to improve the predictive value of clinical high-risk criteria and to assist the diagnosis and prognosis in the initial stages of psychosis. Neuroimaging studies have tried to address this issue by examining structural abnormalities in the UHR individuals. Neuroimaging evidence suggests that some neuroanatomical alterations are already present in people at UHR for psychosis whereas others occur during the time of transition, supporting the idea of "late neurodevelopmental disturbance" that occurs during late-adolescence or early adulthood (Pantelis, Yucel et al. 2005).

The results so far are not conclusive due to their heterogeneity; this may at least in part be explained by differences in the samples features (such as socio-demographic characteristics e.g. age, gender), by the diverse inclusion criteria adopted by the different studies, including symptomatology and comorbidities, and by the use of relatively small samples which may have resulted in type I errors (Button, Ioannidis et al. 2013). One of the key problems in the study of the high risk

population is the difficulty in recruiting relatively large samples, which is even more challenging when comparing those UHR individuals who make transition to psychosis (UHR-T, transition) to those who do not (UHR-NT, non-transition).

Another characteristic of past neuroimaging studies investigating structural abnormalities in the UHR population, is that the vast majority of them have used univariate analytical methods that allow statistical inferences at group level. However clinicians have to take decisions based on the single individual in their day-to-day clinical practice, and therefore the translational impact of these results is limited. Univariate approaches are well suited to detect focal abnormalities at the group level; however, differences in brain anatomy in the high risk population are likely to be relatively subtle and widespread. Interestingly, findings from past studies suggest that structural alterations in the UHR population are not confined to single cortical regions nor spread in the entire brain but they are expressed within a distributed network, which mainly encompasses prefrontal and temporal regions (Smieskova, Fusar-Poli et al. 2010, Egerton 2011, Fusar-Poli, Radua et al. 2012). Univariate approaches also involve multiple testing and the subsequent correction for multiple comparisons; therefore they may be too conservative and not sensitive enough to detect alterations that are expressed at network level rather than in distinct areas. Multivariate approaches, such as Support Vector Machines (SMV), consider multiple voxels simultaneously and between-voxels correlations rather then each voxel independently, and as such may be better suited to detect network abnormalities in the population at risk for psychosis (Lao, Shen et al. 2004, Norman, Polyn et al. 2006). In addition, multivariate approaches allow inferences to be made at the individual level, and therefore they carry greater translational potential in day-to-day clinical practice.

SVM is a 'supervised' multivariate classification method that allows the binary categorization of individuals in distinct groups or classes (e.g. patients and healthy controls) based on the detection of patterns in high-dimensional data, such as structural brain images. The term 'supervised' refers to the training step in which the algorithm learns to differentiate between two groups or classes (Vapnik 1999). SVM comprises a training phase and a testing phase. During the training phase, an algorithm that identifies the brain regions that better distinguish the two groups under investigation (e.g. patients and healthy controls) is developed. The aim is for the algorithm to predict the labels from a set of previously unseen data (Burges 1998). In the testing phase, a new subject is recognised as belonging to one of the classes based on the decision function previously learnt (Schrouff, Rosa et al. 2013).

During the last two decades, machine learning multivariate approaches have been successfully applied to the investigation of neuroanatomical abnormalities in a number of psychiatric and neurological disorders (Orru, Pettersson-Yeo et al. 2012). In particular, SVM has been employed in diagnostic studies, studies on prediction of treatment response and studies on prediction of disease onset (Orru, Pettersson-Yeo et al. 2012). SVM has been employed with the UHR population both to test its ability to discriminate UHR and healthy controls, and within the UHR group, UHR-T and UHR-NT. Relatively recent studies have reported high levels of accuracy in the prediction of group membership, effectively distinguishing UHR from healthy controls, and UHR-T from UHR-NT subjects (Koutsouleris, Meisenzahl et al. 2009, Koutsouleris, Borgwardt et al. 2012). For instance, Koutsouleris and colleagues (2009) using structural MRI have successfully distinguished the UHR-T group from the healthy control group with an accuracy of 94%, UHR-NT from healthy controls with an accuracy of 86% and UHR-T from UHR-NT with an accuracy of 82% (Koutsouleris, Meisenzahl et al. 2009). Similar results from an independent data set study showed

classification accuracy respectively of 92.3% for healthy controls and UHR-T, of 66.9%, for healthy controls and UHR-NT and of 84.2%, for UHR-T and UHR-NT (Koutsouleris, Borgwardt et al. 2012). In addition, UHR-T could be successfully distinguished from first episode psychosis with a 80% accuracy (Borgwardt, Koutsouleris et al. 2013). Finally, when combining two independent data sets, SVM could successfully distinguish UHR-T from UHR-NT with 80.3% accuracy (Koutsouleris, Riecher-Rossler et al. 2014). No studies so far have assessed the predictive value of cortical thickness in the UHR population. While these initial results suggest this approach holds some promise, the studies published so far have used relatively small and selected groups of subjects; more evidence must therefore be collected from larger and more representative samples in order to evaluate the generalizability of the findings and their translational potential in clinical practice.

In the work presented in this chapter, Support Vector Machines (SVM) was applied to a large multi-centre sample of UHR individuals and matched healthy controls. Whole-brain magnetic resonance images were acquired from individuals at UHR for psychosis and healthy controls at four psychiatric research centres in London, Basel, Melbourne and Munich. At each site, subjects were scanned at first clinical presentation, and followed clinically for an average of at least two years, so that they could be subcategorized according to the clinical outcome (i.e. UHR-T and UHR-NT). The MRI data from each site were combined to form a large UHR sample, which was subdivided into subjects who had developed psychosis and subjects who had not.

The main objective of this work was to investigate the predictive value of neuroanatomical MRI scans in a relatively large sample of individuals at UHR for psychosis and matched healthy controls. The first aim of the work described in this chapter was to assess whether a multivariate pattern classification approach, such as SVM, is able to distinguish with a statistically significant level of accuracy, between UHR and healthy

controls, using neuroanatomical data such as gray matter volume and cortical thickness. The second aim was to assess whether SVM is able to distinguish with a significant level of accuracy, between UHR-T and UHR-NT, using gray matter volume and cortical thickness information.

Hypothesis:

1) Structural imaging data (i.e. gray matter volume and cortical thickness) can be used to discriminate between UHR participants and healthy controls with statistically significant accuracy.
2) Structural imaging data can be used to discriminate between UHR individuals who did and did not make a transition to psychosis with statistically significant accuracy.

5.2. Methods

5.2.1. Design

A multi-centre cross-sectional study design was employed comparing 167 individuals at UHR for psychosis and 150 matched healthy controls and, within the UHR group, 50 individuals who developed psychosis at follow-up and 117 who did not.

5.2.2. Inclusion criteria

Inclusion and exclusion criteria for UHR and HC groups were reported in Chapter 2, sections 2.2. and 2.2.2.

5.2.3. Sample

All the UHR subjects were recruited from four specialised clinical services for young individuals at high risk of psychosis in London (i.e. 1.5T), Basel, Melbourne and Munich. Recruitment details are reported in Chapter 2, sections 2.2. and 2.2.2. MRI structural data were thus combined using the four samples employed also for the study described in Chapter 4. Individuals at UHR were followed up for about 30.6 months (mean follow up period; SD=10.4) subsequent to scanning, and

50 of the original group (29.5%) of UHR developed psychosis (UHR-T), while 117 did not (UHR-NT). At each site, healthy controls from the same geographical area as the UHR subjects were recruited through local advertisement. Each site yielded a dataset that included an UHR-T group, an UHR-NT group and a healthy control group.

5.2.4. Image acquisition

At all four sites, volumetric MR images were acquired using scanner field strength of 1.5T and a T1-weighted protocol. Two sites used General Electric scanners, and two used Siemens scanners. The details of the image acquisition sequences varied between scanners, as reported in Chapter 2, section 2.3.2.

5.3. Data analysis

5.3.1. Sociodemographic data

Differences in demographics and clinical profile between groups were examined using one-way analysis of variance (ANOVA) for parametric data, and a chi square test for non-parametric data, as implemented in the Statistical Package for the Social Sciences (SPSS 17.0 for Windows, IBM SPSS statistics 18.0 and 19.0 for windows and mac), (see **Table 4.2.**, Chapter 4). Sociodemographic characteristics of the study samples by site are reported in Appendix 3 (Table A3.2.).

5.3.2. Analysis of neuroanatomical data

The analysis of the MRI data comprised of three main components. Firstly, the unified segmentation procedure (Ashburner and Friston 2005) implemented in SPM8 (http://www.fil.ion.ucl.ac.uk/spm) was used to segment all the images into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) partitions. Secondly, the images were then pre-processed using two alternative approaches that allowed the extraction of information on gray matter volume and cortical thickness respectively. Thirdly, Support Vector Machine (SVM) was

used as implemented in the Pattern Recognition for Neuroimaging Toolbox (PRoNTo; http://www.mlnl.cs.ucl.ac.uk/pronto/) (Schrouff, Rosa et al. 2013). A detailed description of each component is reported below, in Chapter 3, section 3.2.3.2., and Chapter 4, section 4.2.3.2.

5.3.2.1. Creation of voxel-based gray matter volume maps

Preprocessing of gray matter images is described in details in Chapter 3, section 3.2.3.2. After the preprocessing smoothed, modulated, normalised data were obtained and were subsequently used for the statistical analyses.

5.3.2.2. Creation of voxel-based cortical thickness maps

Preprocessing for the creation of cortical thickness maps is described in details in Chapter 4, section 4.2.3.2. The procedure results in smoothed, wraped, voxel-based cortical thickness (VBCT) maps that were subsequently used for the statistical analyses.

5.3.2.3.Multivariate SVM analyses

A linear SVM was used to classify UHR participants and healthy controls, and within the UHR group, UHR-T and UHR-NT, on the basis of their brain structure. After the preprocessing, the segmented images were entered (gray matter volume and cortical thickness separately) into SVMs as implemented in the PRoNTo software package running under Matlab 7.1 (Math Works, Natick, MA, USA) to assess the diagnostic value of whole brain structural images (Schrouff, Rosa et al. 2013). For each comparison subjects were used to construct samples for the classifier, with each individual scan treated as a data point located in high dimensional space and assigned the operator a given class. Each classifier was embedded in a leave-one-out-cross-validation (LOOCV) framework, where all but one pair of images (i.e. one subject for each class) were excluded from the overall group and used as training data for the classifier and the remaining images as test data. During the training phase the algorithm finds the optimal-separating-hyperplane (OSH) that divides the examples in the input space

according to their class labels. The OSH is constructed by finding the hyperplane that maximizes the distance between the samples that are the most difficult to classify. The rationale is that maximizing the separation between ambiguous data points, the classifier will also accurately classify previously unseen data. The decision function was used to classify the test set of images. Results are reported in the terms of balanced accuracy that takes into account unbalanced designs. The balanced accuracy of the classifier was calculated by taking the mean of its sensitivity (i.e. proportion of subjects correctly classified as belonging to a certain class) and specificity (i.e. proportion of subjects correctly classify as not belonging to a certain class) across all LOOCV folds. In order to determine the statistical significance of the accuracy, a permutation test was performed whereby subjects were randomly assigned to a class and the LOOCV cycle repeated. A 1000-time repetition provides a distribution of accuracies reflecting the null hypothesis that the classifier to not exceed the chance. This is subsequently used to estimate the p-value for the significance of the classification algorithm, by counting the number of times the permuted accuracy was greater than or equal to the true accuracy and dividing it by 1000. For each comparison a multivariate discrimination map was produced visualizing each voxel's vector score ($\mathbf{w_i}$), representing its relative contribution in defining the OSH, displaying the pattern of regions able to discriminate each group. Consistently with previous studies an arbitrary threshold was also applied to each map such that only voxels with a $\mathbf{w_i}$ value above 30% of the maximum absolute value were shown (Gong, Wu et al. 2011, Gong, Li et al. 2013). For all classifiers, a linear kernel was used and the SVM parameter C was fixed to unity.

5.4. Results

5.4.1. Sociodemographic data

No statistically significant differences were noted among the UHR-T, UHR-NT, and control groups in age, gender, ethnicity, handedness and medication (**Table 4.2.** Chapter 4).

5.4.2. Multivariate SVM analyses

Results from each comparison are reported below. For ease of visualisation, tables and figures were created using an arbitrary 70% threshold for all SVM derived weight maps, showing those regions with weight vector values in the top 30% of the absolute maximum weight vector values across all regions. These values represent the relative contribution of each voxel to the decision function, in the context of every other voxel.

5.4.2.1. SVM classification of HC and UHR

Using gray matter volume information, SVM was able to discriminate between UHR individuals and healthy controls with a balanced accuracy of 56.8% (specificity: 54%; sensitivity: 59.6%), which was statistically significant (p = 0.0310). The use of an arbitrary threshold corresponding to 30% of the maximum weight vector score showed that the prediction appeared to be based on a distributed pattern of gray matter regions including, in particular, the inferior, superior and middle frontal gyrus, middle temporal gyrus, lingual gyrus, precuneus, cuneus, inferior parietal lobule and angular gyrus (see **Table 5.1.** and **Figure 5.1.**). Using cortical thickness information, SVM was able to discriminate between UHR individuals and healthy controls with a balanced accuracy of 54% (specificity: 48.6%; sensitivity: 59.3%), which did not reach statistical significance level (p = 0.1240).

Figure 5.1.

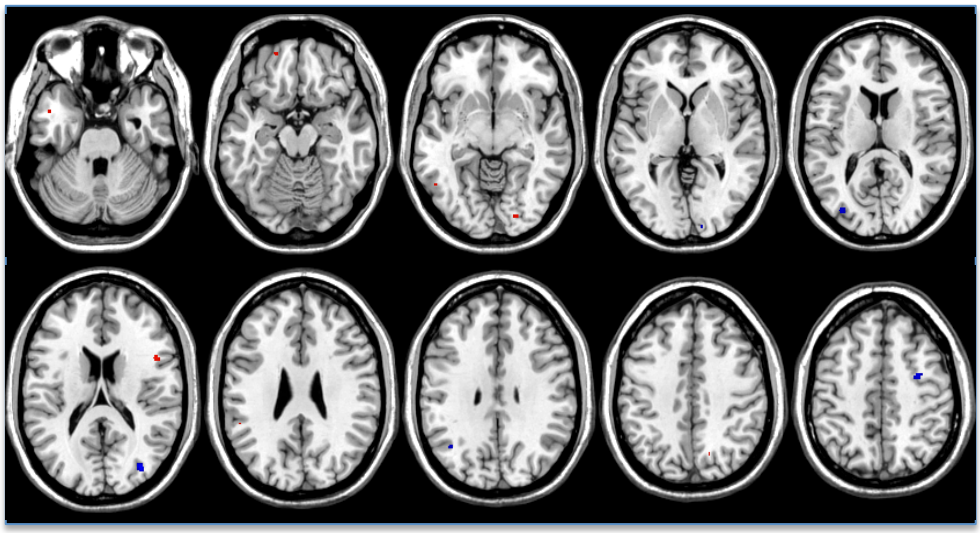

Figure 5.1. Multivariate map SVM classification of HC and UHR. Gray matter regions that showed the highest discriminative value for the comparison between UHR and healthy controls. Regions were identified by setting the threshold to the top 30% of the maximum absolute weight vector score. Red indicates higher values in UHR group and blue indicates higher values in the HC group.

Table 5.1. SVM classification of HC and UHR

Region	**Number of Voxels**	**MNI Coordinate (x, y, z)**	w_i
Gray Matter Regions with Positive w_i Scores			
Inferior Frontal Gyrus	37	48 12 19.5	0.00755
Middle Temporal Gyrus	21	-51 -58.5 -6	0.00751
	6	-46.5 4.5 -28.5	0.0065
Lingual Gyrus	14	21 -85.5 -7.5	0.00677
	5	12 -73.5 -4.5	0.00636
Superior Frontal Gyrus	8	-19.5 54 -16.5	0.0065
Precuneus	3	15 -66 42	0.0062
Inferior Parietal Lobule	2	-52.5 -42 27	0.0062
Gray Matter Regions with Negative w_i Scores			
Middle Temporal	50	33 -78 18	0.00965

Gyrus			
Middle Frontal Gyrus	33	31.5 -3 48	0.00843
	1	33 19.5 43.5	0.00692
Middle Occipital Gyrus	27	-33 -81 13.5	0.00771
	4	28.5 -93 -3	0.00693
Angular Gyrus	8	-40.5 -61.5 31.5	0.00724
Cuneus	2	13.5 -94.5 1.5	0.00684

Table 5.1. Neuroanatomical regions with a weight vector score in the 30% of the maximum weight vector score across all regions for gray matter volume. $\boldsymbol{w_i}$, and MNI coordinates refer to the peak weight vector score in each cluster. Abbreviations: MNI, Montreal Neurological Institute: wi, weight vector score indicating the relative contribution of each voxel to the decision function.

5.4.2.2. SVM classification of HC and UHR-T

Using gray matter volume information, SVM was able to discriminate between UHR individuals who develop psychosis at follow up and healthy controls with a balanced accuracy of 51.3% (specificity: 84.7%; sensitivity: 18%), which did not reach statistical significance (p = 0.3430). Using cortical thickness information, SVM was able to discriminate between UHR individuals who develop psychosis at follow up and healthy controls with a balanced accuracy of 53.9% (specificity: 89.9%; sensitivity: 18%;), which did not reach statistical significance (p = 0.1080).

5.4.2.3. SVM classification of HC and UHR-NT

Using gray matter volume information, SVM was able to discriminate between UHR individuals who did not develop psychosis and healthy controls with a balanced accuracy of 59.8% (specificity: 67.3%; sensitivity: 52.3%), which was statistically significant (p = 0.0050). The use of an arbitrary threshold corresponding to the 30% of the maximum weight vector score showed that the prediction appeared to be based on a distributed pattern of gray matter regions including, in particular, the middle frontal gyrus, lingual gyrus, middle occipital

gyrus, cuneus, precentral and postcentral gyrus, angular gyrus, middle and superior temporal gyrus and sub-gyral region (see **Table 5.2.** and **Figure 5.2.**). Using cortical thickness information, SVM was able to discriminate between UHR individuals who did not develop psychosis and healthy controls with a balanced accuracy of 51.06 % (specificity: 62.2%; sensitivity: 41%), which did not reach statistical significance (p = 0.3330).

Figure 5.2.

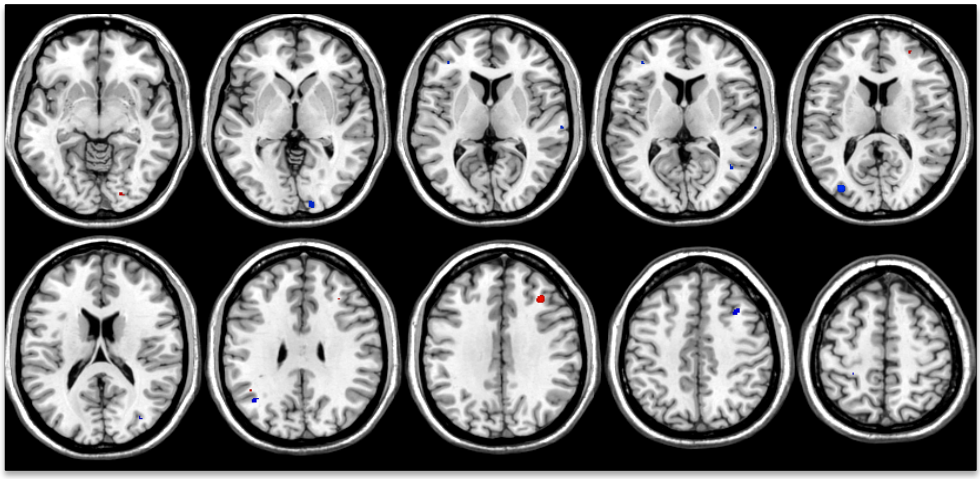

Figure 5.2. Multivariate map SVM classification of HC and UHR-NT. Gray matter regions that showed the highest discriminative value for the comparison between UHR-NT and healthy controls. Regions were identified by setting the threshold to the top 30% of the maximum absolute weight vector score. Red indicates higher values in UHR-NT group and blue indicates higher values in the HC group.

Table 5.2. SVM classification of HC and UHR-NT

Region	**Number of Voxels**	**MNI Coordinate (x, y, z)**	w_i
Gray Matter Regions with Positive w_i Scores			
Middle Frontal Gyrus	42	33 30 33	0.0088
Lingual Gyrus	1	21 -87 -7.5	0.00665
Gray Matter Regions with Negative w_i Scores			
Middle Occipital Gyrus	74	-33 -81 13.5	0.0089

Cuneus	42	15 -96 0	0.00739
Precentral Gyrus	39	33 19.5 45	0.00796
Angular Gyrus	38	-40.5 -61.5 30	0.00768
Middle Temporal Gyrus	7	42 -60 9	0.00687
	4	33 -78 18	0.00677
Sub-Gyral	5	-37.5 40.5 9	0.00673
	1	-31.5-43.5 40.5	0.00649
Superior Temporal Gyrus	4	64.5 -22.5 7.5	0.00671
Postcentral Gyrus	2	-27 -37.5 55.5	0.00663

Table 5.2. Neuroanatomical regions with a weight vector score in the 30% of the maximum absolute weight vector score across all regions for gray matter volume. $\boldsymbol{w_i}$, and MNI coordinates refer to the peak weight vector score in each cluster. Abbreviations: MNI, Montreal Neurological Institute: wi, weight vector score indicating the relative contribution of each voxel to the decision function.

5.4.2.4. SVM classification of UHR-NT and UHR-T

Using gray matter volume information, SVM was able to discriminate between UHR individuals who developed psychosis at follow-up and UHR individuals who did not with a balanced accuracy of 52.7% (specificity: 77.5%; sensitivity: 28%), which did not reach the statistical significance (p = 0.2410). Using cortical thickness information, SVM was able to discriminate between UHR individuals who developed psychosis at follow-up and UHR who did not with a balanced accuracy: 56.5% (specificity: 82.9%; sensitivity: 30%), which was statistically significant (p < 0.0470). The use of an arbitrary threshold corresponding to the 30% of the maximum weight vector score showed that the prediction appeared to be based on a distributed pattern of cortical regions including, in particular, the middle temporal gyrus, subcallosal gyrus, medial and inferior frontal gyrus, lingual gyrus, parahippocampal gyrus, orbital gyrus, fusiform gyrus and inferior occipital gyrus (see **Table 5.3.** and **Figure 5.3.**).

Figure 5.3.

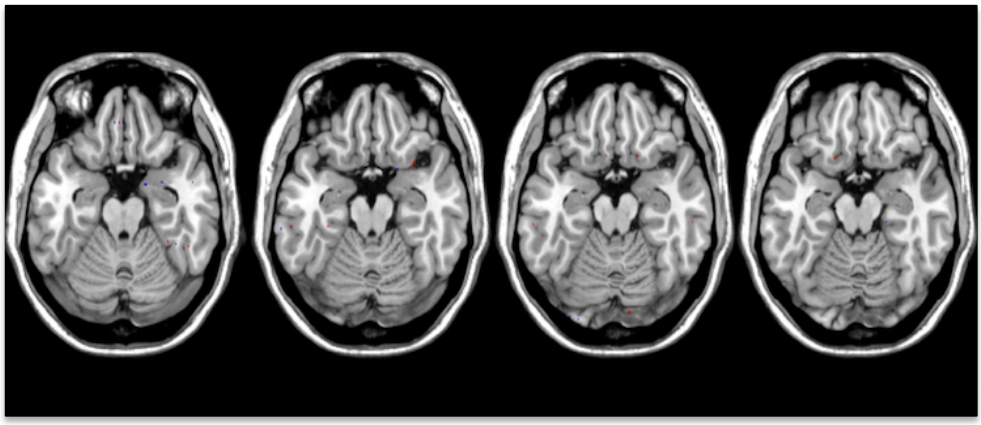

Figure 5.3. Multivariate map SVM classification of UHR-NT and UHR-T. Cortical regions that showed the highest discriminative value for the comparison between UHR-T and UHR-NT. Regions were identified by setting the threshold to the top 30% of the maximum absolute weight vector score. Red indicates higher values in UHR-T group and blue indicates higher values in the UHR-NT group.

Table 5.3. SVM classification of UHR-NT and UHR-T

Region	**Number of Voxels**	**MNI Coordinate (x, y, z)**	w_i
Cortical Thickness Regions with Positive w_i Scores			
Middle Temporal Gyrus	2	-60 -28.5 -18	0.0327
Subcallosal Gyrus	1	-16.5 16.5 -16.5	0.028
Medial Frontal Gyrus	1	-6 18 -18	0.0282
	1	1.5 15 -18	0.0303
	1	6 10.5 -19.5	0.0354
Inferior Frontal Gyrus	1	16.5 18 -18	0.0265
	1	30 10.5 -19.5	0.0294
	1	31.5 13.5 -19.5	0.0298
Middle Temporal Gyrus	1	57 -27 -18	0.0281
Lingual Gyrus	1	10.5 -88.5 -18	0.0263
Parahippocampal Gyrus	1	-31.5 -30 -19.5	0.0368
Orbital Gyrus	1	-3 40.5 -21	0.0333

Fusiform Gyrus	1	33 -40.5 -21	0.0265
	1	48 -43.5 -21	0.0287
Cortical Thickness Regions with Negative w_i Scores			
Parahippocampal Gyrus	1	21 -27 -16.5	0.0266
	1	28.5 0 -21	0.0294
	1	16.5 -1.5 -21	0.0278
Medial Frontal Gyrus	1	-1.5 15 -18	0.0329
Inferior Occipital Gyrus	1	-33 -91.5 -18	0.0258
	1	-27 -93 -18	0.0366
Inferior Frontal Gyrus	1	19.5 9 -19.5	0.0292
Orbital Gyrus	1	-6 40.5 -21	0.0282
	1	-1.4 40.5 -1.4	0.0289
Middle Temporal Gyrus	1	51 0 -21	0.0266
Fusiform Gyrus	1	39 -42 -21	0.0258

Table 5.3. Neuroanatomical regions with a weight vector score in the 30% of the maximum weight vector score across all regions for cortical thickness. $\boldsymbol{w_i}$, and MNI coordinates refer to the peak weight vector score in each cluster. Abbreviations: MNI, Montreal Neurological Institute: wi, weight vector score indicating the relative contribution of each voxel to the decision function.

5.5. Discussion

The assessment and detection of individuals at UHR for psychosis are complex due to the fact that many of the prodromal symptoms are non-specific, since they overlap both with symptoms present in other psychiatric disorders (Ellison-Wright and Bullmore 2010, Tschoeke, Steinert et al. 2014) and psychotic-like-experiences that may occur in the general population (van Os, Hanssen et al. 2000, van Os, Linscott et al. 2009). To date it is not possible to predict which individuals who meet the criteria for UHR for psychosis will make transition to psychosis based on their clinical presentation; therefore all individuals

accessing early detection services are offered the same therapeutic options. However, the limited amount of available clinical resources highlights the need for alternative tools that can aid diagnostic and prognostic processes. Tools such as SVM offer the potential of assisting these processes.

The first objective of this work was to determine whether machine learning and structural imaging data, specifically gray matter volume and cortical thickness, would allow classification of a large multi-centre sample of UHR participants and healthy controls with statistically significant accuracy. UHR individuals could be successfully distinguished from healthy controls using gray matter volume information (balanced accuracy = 56.8%, p = 0.0310). In contrast, cortical thickness information did not allow an accurate discrimination of UHR from healthy controls (balanced accuracy = 54%, p = 0.1240). The gray matter regions that held the highest discriminative values were distributed in a bilateral widespread network of regions including the inferior, superior and middle frontal gyrus, middle temporal gyrus, lingual gyrus, precuneus and cuneus, inferior parietal lobule and angular gyrus (**Table 5.1.** and **Figure 5.1.**).

The application of SVM to gray matter volume further allowed an accurate classification of UHR-NT individuals from healthy controls (balanced accuracy = 59.8%, p = 0.0050). The gray matter regions that held the highest discriminative values were also distributed in a bilateral widespread network of regions including the middle frontal gyrus, lingual gyrus, middle occipital gyrus, cuneus, precentral and postcentral gyrus, angular gyrus, middle and superior temporal gyrus and sub-gyral region (**Table 5.2.** and **Figure 5.2.**). In contrast, gray matter volume information did not allow a successful discrimination of UHR-T from healthy controls (balanced accuracy = 51.3%, p = 0.3430). Cortical thickness did not allow a successful discrimination either of UHR-T or of UHR-NT from healthy controls (HC vs UHR-T balanced

accuracy 53.9%, p = 0.1080; HC vs UHR-NT balanced accuracy 51.06%, p = 0.3330).

The second objective of this work was to determine whether machine learning and structural imaging data, specifically gray matter volume and cortical thickness, would allow discrimination between UHR individuals who did and did not make transition at follow-up with statistically significant accuracy. Findings revealed that UHR-T could be successfully distinguished from UHR-NT using cortical thickness information (balanced accuracy = 56.5%, p = 0.0470). The cortical areas that held the highest discriminative values were distributed in a bilateral widespread network of regions including the middle temporal gyrus, subcallosal gyrus, medial and inferior frontal gyrus, lingual gyrus, parahippocampal gyrus, orbital gyrus and fusiform gyrus (**Table 5.3.** and **Figure 5.3.**). In contrast, gray matter volume did not allow a significant classification of UHR-T and UHR-NT (balanced accuracy = 52.7%, p = 0.2410).

While the results of this work provide further evidence for the implication of the above regions in psychosis, it has to be noted that when structural data are analysed using a multivariate approach the findings cannot be interpreted in terms of greater or lower volume or thickness in one group compared to the other. This is because the regions identified in each group are those that are the most important in defining group membership; this can be the case either because of a difference in volume between groups in that region or because of a difference in the correlation between that region and other areas of the brain. Therefore, the regions identified in the present study should be interpreted as parts of a spatially distributed pattern of neuroanatomical alterations rather than as independent areas. In addition, it should be noted that these regions were identified using an arbitrary threshold of 30% based on previous studies (Gong, Wu et al. 2011, Gong, Li et al. 2013), and that prediction of class membership was

to some extent informed by all voxels in the brain since no feature extraction was employed.

Interestingly, gray matter volume information allowed an accurate discrimination of UHR from healthy controls and UHR who did not make transition from healthy controls, whereas cortical thickness information allowed the discrimination of UHR who made transition from those who did not. These findings suggest that network abnormalities in gray matter volume could be more associated to vulnerability to psychosis while cortical network abnormalities could be more associated to transition to psychosis, supporting the idea that cortical thickness and gray matter volume offer distinct and complementary information. This is consistent with the notion that cortical thickness and surface area are genetically and phenotypically independent and that volume, that takes into account both cortical thickness and surface, is more closely related to surface than to cortical thickness (Winkler, Kochunov et al. 2010).

Neither gray matter volume nor cortical thickness allowed the identification of UHR-T from healthy controls. Interestingly, both grey matter volume and cortical thickness showed very high specificity (i.e. proportion of healthy controls correctly classified as not having a condition) and on the contrary very low sensitivity (i.e. proportion of patients correctly classified as having a condition). The disproportion in specificity and sensitivity was also observed in the classification of UHR-NT and UHR-T, both for the successful classification obtained using cortical thickness information and for the unsuccessful one obtained using gray matter volume. This indicates that the two classes were not equally important for the classification and the model favoured respectively the healthy control class and the UHR-NT class over the UHR-T class. This could be partially explained by class imbalance; indeed the UHR-T group has a lower number of subjects (n= 50) compared to healthy controls (n = 150) and UHR-NT (n = 117). SVM

is accurate on moderately imbalanced samples as SVM uses only 'support' vectors to generate the decision function, therefore several subjects that are far from the decision boundary can be potentially removed without affecting the classification (Tang, Zhang et al. 2009). However SVM can be sensitive to high class imbalance and can generate a classifier that is biased towards the larger class, resulting in a high number of false negatives (Tang, Zhang et al. 2009, Lin and Chen 2013).

The accuracy values reported in the preset work are below the 90% mark that has been suggested for routine use of MRI in clinical setting (Kasparek, Thomaz et al. 2011, Iwabuchi, Liddle et al. 2013). While some of the classification accuracies obtained in the present work were statistically significant, it should be noted that their values remain relatively low also when compared to previous machine learning studies conducted in the same population (Koutsouleris, Meisenzahl et al. 2009, Koutsouleris, Schmitt et al. 2009, Koutsouleris, Riecher-Rossler et al. 2014). In particular, in a recent study Koutsouleris and colleagues (2014) combined two of the data sets also included in this work (Koutsouleris, Riecher-Rossler et al. 2014) and were able to correctly predict transition outcomes with a 80% of accuracy. The relatively low level of accuracy in the present work could be in part explained by the challenging combination of more independent data sets. This is consistent with results reported in the literature suggesting that the discrimination performance can decrease when combining data across sites (Kloppel, Stonnington et al. 2008). The present work employed four independent samples that were recruited using different, although comparable, clinical high risk criteria and examined using different MRI protocols. Increasing the number of independent samples could have contributed in increasing the overall variability and consequently in lowering the classification accuracy. In particular, the combination of samples acquired with different acquisition sequences can result in increased variability related to the images properties, such as contrast, signal to noise ratio and voxel size. For example, Kempton

and colleagues have reported a between-sequence variation of 4% in total gray matter volume in six T1 weighted MRI sequences (Kempton, Underwood et al. 2011, Kempton and McGuire 2014). Such a large variation in gray matter volume that is due to between-centre/sequence differences could contribute to mask abnormalities observed in this group of patients.

On the other hand, differences in the recruitment process across centres might have also contributed to increase the heterogeneity in the population itself. This in turn might have decreased the chance of finding reliable network differences that would allow discriminating between groups with a high level of accuracy. The UHR population is heterogeneous in nature. People identified as being at ultra high risk for psychosis might meet different UHR criteria and they might present with diverse symptomatology and comorbidities. For example, the prevalence of Axis I diagnoses, such as depression and anxiety disorders, has been estimated to be around 73% in a large sample of individuals at risk for psychosis (Fusar-Poli, Nelson et al. 2014, Modinos, Allen et al. 2014). Mood and anxiety disorders are characterized by a distinct neurobiology; therefore the prevalence of these symptoms in UHR individuals might further complicate the investigation and identification of neuroanatomical biomarkers that can predict transition outcomes.

In addition, only a subsample of those individuals meeting criteria will go on to develop the full-blown psychotic illness and among these individuals there are differences in the psychopathological trajectory. A recent meta-analysis reported that among those individuals who progress to psychosis, 73% will develop a disorder that lies on the schizophrenia spectrum, while the 11% will develop a mood disorder with psychotic features (Fusar-Poli, Bechdolf et al. 2013). Psychosis is indeed an "umbrella" term that includes heterogeneous illnesses, each of them requiring different treatment approaches. This complicates the

scenario further and potentially adds noise to the data. Thus, a way to improve the predictive value of neuroimaging tools such as SVM could be to adopt a stratification approach which involves sub-categorising individuals that make transition according to their different diagnosis and then applying the resulting decision function to a new independent population to test its validity.

Future studies should aim at combining not only different centres but also different modalities, and clinical, neuropsychological and genetic information, which is expected to increase the predictive accuracy, utility and validity of the method. Moreover, to overcome methodological disadvantages due to differences in MRI acquisition, the ADNI consortium developed a structural MRI sequence that generates an image with similar properties independently from the scanner model and manufacturer (Jack, Bernstein et al. 2008). Multi-centre projects such as EU-GEI (European network of national schizophrenia networks studying gene-environment interaction) (European Network of National Networks studying Gene-Environment Interactions in, van Os et al. 2014) and NAPLS (North American Prodrome Longitudinal Study), (Cannon, Cadenhead et al. 2008) that have adopted this approach, will be able to optimize the investigation of large samples deriving form the data-pooling and at the same time to minimise between-centre differences.

5.5.1 Limitations

The first limitation of this work lies on the between-centre differences that are likely to originate from scanner-related differences. The field strength of the four scanning machines used was the same for all sites (i.e. 1.5 T), however the four sites have employed different machines and acquisition sequences that might have increased the heterogeneity in the sample. Secondly, the PRoNTo version used in this work did not allow regressing out covariates of no interest such as age, gender, and scanning site. UHR individuals and healthy controls do not differ in

terms of socio-demographic data, which therefore are not likely to have impacted on the results. However, given that it was not possible to remove the effect of scanning site, it cannot be excluded that this could have added noise and consequently lowered the algorithm accuracy in discriminating between classes. Future multi-centre studies should ideally use the same acquisition sequence and calibrate data across centres using phantoms or common subjects to minimise scanner-related differences (Jack, Bernstein et al. 2008). Finally, the different researchers and clinicians at the four centres recruited UHR individuals using different screening instruments. Although the high-risk criteria used in the four sites were comparable, it cannot be excluded that differences in the recruitment phase could have resulted in relatively different groups. Overall these differences might have increased the level of noise in the data that in turn might have significantly decreased the ability of SVM to find the optimal discrimination function.

5.5.2. Conclusions

Developing an alternative tool that can assist the identification of UHR and within this group, the prediction of those more likely to make transition to psychosis, would allow a more selective and safe delivery of the available preventative treatments, which is desirable both from an ethical point of view and for a sensible use of the care resources. The results of the current work show that UHR and healthy controls are distinguishable at the individual level based on information on the gray matter volume. In addition, UHR who made transition to psychosis and those UHR who did not are distinguishable at the individual level using cortical thickness information. Nevertheless, the percentage of accuracy remains low to be meaningfully applied in a real-world clinical setting.

Future multi-centre projects, such as EU-GEI (European Network of National Networks studying Gene-Environment Interactions in, van Os et al. 2014), that are acquiring environmental, genetic, clinical, neuropsychological and neuroimaging information using the same

instruments and the same protocols will be able to clarify the translational potential of machine learning techniques in this population.

6. Using structural neuroimaging to make quantitative predictions of symptom progression in individuals at ultra high risk for psychosis

(Adapted from published paper, Appendix 1, A1.3.)

This study was published in Frontiers in Psychiatry under the title "Using structural neuroimaging to make quantitative predictions of symptom progression in individuals at ultra-high risk for psychosis"

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6.1. Introduction

As discussed in previous chapters, the majority of past neuroimaging studies employed univariate approaches and therefore reported significant effects only at a group level, whereas clinicians treating psychosis have to make decisions about the individual in front of them. Because effects that are significant at a group level do not necessarily permit accurate inferences about individuals, the translational potential of the above findings for everyday clinical practice is unclear. In addition, these studies were conducted using a standard univariate analytical approach in which each voxel is considered independently. This approach is well suited to detect effects that are robust and localised; however, it is not very sensitive to differences that are subtle and highly distributed across the brain. For these reasons, an increasing number of recent studies of psychiatric disorders have adopted an alternative approach based on multivariate machine learning methods (Orru, Pettersson-Yeo et al. 2012, Pettersson-Yeo, Benetti et al. 2013). A key benefit of multivariate machine learning methods is that they allow one to make predictions that are specific to a given individual, rather than providing an average estimate for a group. This greatly increases the likelihood that the results can be translated into a tool that is useful in a real world clinical setting. A further benefit of multivariate machine learning methods is that they take into account the inter-relationship between different measures (e.g. gray matter volume across different voxels), and therefore are better suited for detecting subtle and spatially distributed patterns of alteration. The vast majority of multivariate machine learning studies of psychiatric disorders published so far have been limited to categorical decisions such as whether an individual belongs to a patient or control group; whether an individual will respond to treatment or not; or whether an individual will develop a disorder or not (Orru, Pettersson-Yeo et al. 2012). Within this context, studies of the UHR population employing multivariate machine learning methods have typically focused on prediction of

clinical outcome in terms of transition/non-transition to psychosis. For example, Koutsouleris and colleagues (2009) demonstrated that a distributed network of abnormalities in gray matter volume allows prediction of subsequent transition to psychosis with an accuracy of 82% (Koutsouleris, Meisenzahl et al. 2009). This notable finding was replicated in an independent cohort by a subsequent investigation (Koutsouleris, Borgwardt et al. 2012). However, follow-up studies of individuals at UHR have shown substantial heterogeneity in symptom progression both among those who develop psychosis and those who do not (Miller, McGlashan et al. 2002, Velthorst, Nieman et al. 2011). For instance, a recent investigation showed that about 75% of those individuals who do not develop psychosis present with symptoms remission after three years while the remaining 25% are still showing sub-threshold symptoms (Velthorst, Nieman et al. 2011). In addition, even those individuals at UHR who show full or partial remission of positive symptoms remain at a lower level of functioning compared to non-psychiatric comparison individuals (Addington, Cornblatt et al. 2011). Another study reported that only 30% of those individuals who do not develop psychosis experience a full symptomatic and functional recovery (Schlosser, Jacobson et al. 2012). Despite the high degree of heterogeneity in clinical outcome beyond and above transition of psychosis, none of the multivariate machine learning studies of the UHR population published so far have focussed on quantitative changes in symptomatology.

Here I sought to expand the existing literature by investigating the potential of structural MRI for predicting the course of clinical symptomatology at 2-year follow-up in UHR individuals using Relevance Vector Regression (Tipping 2001). The advantage of RVR relative to other multivariate machine learning techniques, such as Support Vector Machine (Orru, Pettersson-Yeo et al. 2012), is that it allows the quantitative prediction of a variable of interest (e.g. a patient's score on a clinical scale) at individual level, without the need

for a discrete categorical decision (e.g. patients vs. controls). In recent years, RVR has been successfully used in several neuroimaging studies of healthy people (Franke, Ziegler et al. 2010, Mwangi, Hasan et al. 2013) and patients with psychiatric (Mwangi, Matthews et al. 2012, Gong, Li et al. 2014) or neurological disorders (Stonnington, Chu et al. 2010). It is therefore hypothesised that the application of RVR to neuroanatomical data, particularly gray matter volume and cortical thickness, would allow quantitative prediction of symptom progression at individual level with statistically significant accuracy.

6.2. Methods

6.2.1. Sample

The total sample consisted of 40 UHR subjects recruited at first presentation from consecutive referrals to the Outreach and Support in South London (OASIS) service in London, UK (Fusar-Poli, Byrne et al. 2013). Subsequent to MRI scanning, the UHR subjects were monitored for at least 2 years. Over the 2-year follow-up, 7 UHR individuals developed psychosis and the remaining 33 did not. Transition to psychosis during the follow-up period was established according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria based on clinical consensus between at least two experienced psychiatrists. Most of the UHR group (31/44 70%) were naïve to antipsychotics at the time of scanning; the remaining 13 (30%) had been exposed to antipsychotics for an average of 9.7 weeks (SD=13.3).

6.2.2. Sociodemographic data and clinical measures

Socio-demographic measures included age, gender and years of education. Clinical symptoms were assessed in all participants at the time of scanning and at 2-year follow-up using the Positive and Negative Syndrome Scale (PANSS)(Kay, Fiszbein et al. 1987). Symptoms in the UHR participants were also assessed using the Comprehensive

Assessment of At-Risk Mental States (CAARMS) (Yung, Yuen et al. 2005). Socio-demographic and clinical variables were analysed using Student's t-test for continuous data and a chi square test for ordinal data. These statistical analyses were performed using the Statistical Package for the Social Sciences 19.0 (SPSS 19.0 for Windows, Chicago, Illinois, USA).

6.2.3. Acquisition of neuroanatomical data

Neuroanatomical images were acquired using a 1.5 T GE NV/I Signa LX Horyzon system (General Electric, Milwaukee, WI, USA) at Mapother House, Maudsley Hospital. Details of the image acquisition sequence are reported in Chapter 2, section 2.3.2.

6.2.4. Analysis of neuroanatomical data

The analysis of the MRI data comprised of 3 main components. Firstly, the unified segmentation procedure (Ashburner and Friston 2005) implemented in SPM8 (http://www.fil.ion.ucl.ac.uk/spm) was used to segment all the images into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) partitions. I then pre-processed the images using two alternative approaches that allowed me to extract information on gray matter volume and cortical thickness respectively. Secondly, I used multivariate Relevance Vector Regression (Tipping 2001) as implemented in the Pattern Recognition for Neuroimaging Toolbox (PRoNTo; http://www.mlnl.cs.ucl.ac.uk/pronto/). Thirdly, I performed a standard univariate analysis as implemented in Statistical Parametric Mapping (SPM8) software. A detailed description of each component is reported below, in Chapter 3, section 3.2.3.2., and Chapter 4, section 4.2.3.2.

6.2.4.1. Creation of voxel-based gray matter volume maps

Preprocessing of gray matter images is described in details in Chapter 3, section 3.2.3.2. After the preprocessing smoothed, modulated,

normalised data were obtained and were subsequently used for the statistical analyses.

6.2.4.2. Creation of voxel-based cortical thickness maps

Preprocessing for the creation of cortical thickness maps is described in details in Chapter 4, section 4.2.3.2. The procedure results in smoothed, wraped, voxel-based cortical thickness (VBCT) maps that were subsequently used for the statistical analyses.

6.2.4.3. Multivariate RVR analyses

I examined the relationship between brain structure and changes in PANSS total score from baseline to two years follow-up using multivariate RVR as implemented in PRoNTo (http://www.mlnl.cs.ucl.ac.uk/pronto/) running under Matlab (Mathworks, 2010 release), (Schrouff, Rosa et al. 2013). This method has been described elsewhere (Gong, Li et al. 2014). In brief, RVR is a sparse kernel learning multivariate regression method set in a fully probabilistic Bayesian framework. Under this framework, a zero-mean Gaussian prior is introduced over the model weights, governed by a set of hyperparameters – one for each weight. The most probable values for these hyperparameters are then iteratively estimated from the training data, with sparseness achieved due to the posterior distributions of many of the weights peaking sharply around zero; those training vectors associated with non-zero weights are referred to as 'relevance' vectors. The optimised posterior distribution over the weights can then be used to predict the target value (e.g. PANSS score) for a previously unseen input vector (e.g. cortical thickness map) by computing the predictive distribution (for a more in-depth and detailed description see Tipping, 2001 (Tipping 2001).

In the current study, the input vectors (i.e. each subjects gray matter volume and cortical thickness maps, respectively) were mean centred using the training data, and an estimate for the model's generalizability obtained via leave-one-out cross validation, indexed using the Pearson

correlation coefficient and mean square error (MSE) between actual and predicted difference between baseline and follow–up on PANSS total scores. The significance of both the correlation coefficient and the MSE score was estimated using a permutation test whereby the input-target data were randomly paired and the RVR re-run 1000 times. This created a distribution of correlation and MSE values reflecting the null hypothesis that the model did not exceed chance. The number of times the permuted value was greater than (or with respect to MSE values, less than), or equal to, the true value, was then divided by 1000 providing an estimated p-value for both the correlation coefficient and observed MSE. For ease of visualisation, a table was also created using an arbitrary 70% threshold for all successful RVR derived weight maps, showing those regions with weight vector values in the top 30% of the absolute maximum weight vector values across all regions. These values represent the relative contribution of each voxel to the regression function, in the context of every other voxel.

6.2.4.4. Univariate SPM analyses

I also examined the relationship between brain structure and changes in PANSS total score from baseline to follow-up using a standard, univariate approach. A multiple regression model was performed in SPM8 software to identify any voxels in the gray matter volume and cortical thickness maps respectively that showed a significant association with PANSS total scores. Statistical inferences were made at $p < 0.05$ (corrected for multiple comparisons using Family-Wise Error (FWE)). For completeness, when no significant effects were found, I also examined trends at $p < 0.001$uncorrected.

6.3. Results

6.3.1. Sociodemographic data and clinical measures

Socio-demographic and clinical variables are reported in **Table 6.1.** for all participants as well as for the sub-groups that did and did not make transition to psychosis separately. It can be seen that, on average,

participants showed clinical improvement at follow-up relative to baseline (t = -2.555; p = 0.015; df =39). Examination of the subject-specific scores revealed that 26 individuals improved, 3 remained stable and 11 worsened over the two years follow-up time. No significant association were found between the change in PANSS total scores from baseline to follow-up and antipsychotic medication (t = -0.269, df = 38, p = 0.789).

Table 6.1. Demographic and clinical variables by group

Characteristics	**UHR** (N = 40)	**UHR-NT** (N = 33)	**UHR-T** (N = 7)	**Group Comparison**
Age (mean, SD)	23.90 (4.50)	24.06 (4.61)	23.14 (4.18)	$t_{(38)}$ = 0.48 p = 0.63
Gender (Male / Female)	25/15	20/13	5/2	X^2_1 = 0.29 p = 0.59
Years of Education	12.82 (2.31)	12.88 (2.31)	12.50 (2.51)	$t_{(36)}$ = 0.36 p = 0.72
PANSS total baseline	53.30 (14.95)	50.27 (12.02)	67.57 (19.85)	$t_{(38)}$ = -3.06 p = 0.004
PANSS total follow-up	46.50 (13.34)	43.61 (10.25)	60.14 (18.27)	$t_{(38)}$ = -3.34 p = 0.002
Difference PANSS follow-up/PANSS baseline	-6.80 (16.83) $t_{(39)}$ = -2.555 p = 0.015	6.67 (15.16)	7.43 (24.78)	$t_{(38)}$ = -0.10 p = 0.91

Table 6.1. Data reflect mean (and standard deviation). Abbreviations: PANSS, Positive and Negative Syndrome Scale

6.3.2. Multivariate RVR analyses

The application of RVR to whole-brain cortical thickness images allowed quantitative prediction of symptom progression with statistically significant accuracy (correlation = 0.34, p-value = 0.026; Mean Squared-Error = 249.63, p = 0.024, **Figure 6.1.**). The use of an arbitrary threshold corresponding to the 30% of the maximum weight vector score showed that the prediction appeared to be based on a distributed pattern of cortical thickness including, in particular, the left insular cortex and lateral and medial regions of the right temporal

cortex (**Table 6.2**. and **Figure 6.1.**). In contrast, the application of RVR to the whole-brain gray matter volume images did not allow accurate prediction of symptoms progression (correlation = 0.14, p = 0.627; Mean Sum of Squares = 369.50, p = 0.621).

Figure 6.1.

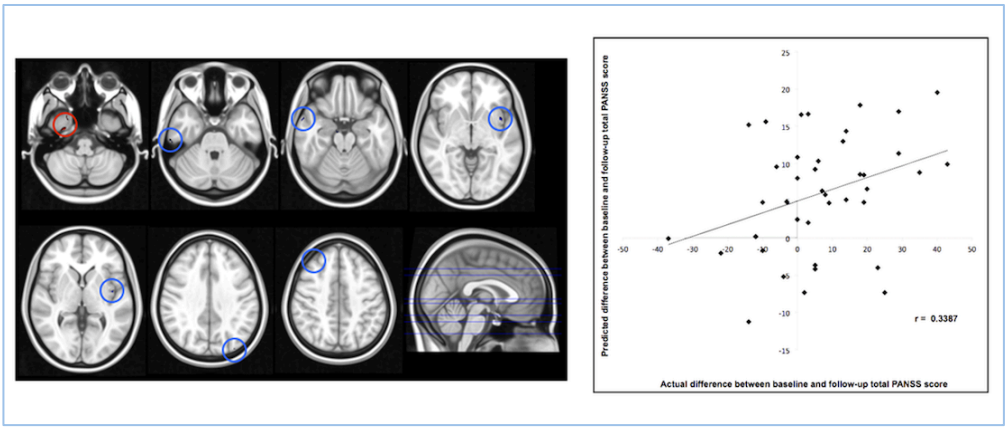

Figure 6.1. Red/Blue circles show voxels with a weight score in the 30% of the maximum (range -0.011608 – 0.026588). Axial Slices (MNI) Left-Right: -65, -49, -40, -23, -17, 16, 25. Scatter plot showing the predicted difference between baseline and follow up total PANSS score for each subject derived from their cortical thickness data using RVR, versus the actual difference.

Table 6.2. MNI coordinates

Region	**Number of Voxels**	**MNI Coordinate (x, y, z)**	w_i
Regions with Positive w_i Scores			
Temporal			
Right Temporal Fusiform Cortex	42	28.5 -9 -46.5	0.00139
Right Temporal Pole	12	22.5 9 -46.5	0.0111
Regions with Negative w_i Scores			
Subcortical			
Left Thalamus	24	-15 -6 15	-0.00941
Right Posterior Limb of Internal Capsule	19	18 -7.5 16.5	

Table 6.2. Neuroanatomical regions with a weight vector score in the 30% of the maximum weight vector score across all regions for the cortical thickness based RVR used to accurately predict the difference between baseline and follow up total PANSS score. w_i, and MNI coordinates refer to the peak weight vector score in each cluster. Abbreviations: MNI, Montreal Neurological Institute: RVR, relevance rector regression; PANSS, Positive and Negative Syndrome Scale; w_i, weight vector score indicating the relative contribution of each voxel to the regression function.

6.3.3. Univariate SPM analyses

Whole brain analysis of the gray matter volume and cortical thickness data did not detect any regions that showed a significant positive or negative association with the change in PANSS total scores from baseline to follow-up at $p < 0.05$ (FWE corrected). With a less conservative statistical threshold ($p < 0.001$ uncorrected), a number of regions showing a positive association with the change in PANSS total scores were detected. With respect to gray matter volume, the right middle frontal gyrus (MNI coordinates: 39, 15, 37.5; $p = 0.929$; z-score = 3.266) was associated with changes in PANSS scores. With respect to cortical thickness, the right inferior parietal lobule (MNI coordinates: 61.5, -34.5, 25.5; $p = 0.802$; z-score = 3.659), left cingulate gyrus (MNI coordinates: -9, 1.5, 46.5; $p = 0.986$; z-score = 3.340), right middle temporal gyrus (MNI coordinates: 49.5, -63, 4.5; $p = 0.992$; z-score = 3.295) and left insula (MNI coordinates: -34.5, -15, 16.5; $p = 0.998$; z-score = 3.197) were associated with changes in PANSS scores.

6.4. Discussion

At present it is difficult to use clinical data acquired at first clinical presentation to predict subsequent progression of symptoms in individuals at UHR for psychosis. This prevents the selective delivery of potentially preventative interventions to those who are most likely to develop persistent symptoms. Recent studies have shown that the application of multivariate machine learning methods to structural neuroimaging data allows accurate categorical prediction of which individuals at UHR will and will not make transition to psychosis (Koutsouleris, Meisenzahl et al. 2009). However, as discussed in the introduction, within the UHR population there is a substantial heterogeneity in symptom progression above and beyond transition to psychosis (Addington, Cornblatt et al. 2011, Velthorst, Nieman et al. 2011, Schlosser, Jacobson et al. 2012). I therefore examined for the first time whether it is possible to use neuroanatomical information to make accurate quantitative predictions of symptom progression in

individuals at UHR. The present results indicate that the application of RVR to whole-brain cortical thickness MRI data allows quantitative prediction of symptom progression (i.e. both the magnitude and direction of change for each individual) at 2-year follow-up with statistically significant accuracy. This is the first evidence that neuroimaging techniques may inform the clinical assessment of UHR individuals by allowing quantitative estimation of the course of clinical psychopathology. In contrast, gray matter volume did not allow accurate prediction of symptoms progression at individual level despite two previous reports that this information allows categorical prediction of transition to psychosis (Koutsouleris, Meisenzahl et al. 2009, Koutsouleris, Borgwardt et al. 2012).

What is the implication of the differential finding for cortical thickness and gray matter volume? Gray matter volume is thought to depend on local cortical thickness as well as cortical folding and gyrification (i.e. cortical surface area), while cortical thickness does not include measures of local surface (Hutton, De Vita et al. 2008). A recent investigation has also shown that cortical thickness and cortical surface area are genetically and phenotypically independent, and that regional gray matter volume is more closely related to the latter than the former (Winkler, Kochunov et al. 2010). It follows that the two approaches provide complementary information, and that one can be more or less than the other depending on the nature of the neuroanatomical changes being examined. In the context of the present investigation, the fact that symptom progression was predicted by cortical thickness but not gray matter volume indicates that changes in symptomatology are specifically associated with differences in cortical thickness as opposed to cortical folding and gyrification.

Examination of the regions that provided the greatest contribution to prediction of symptom progression identified specific areas amongst others traditionally associated with schizophrenia, namely the right

temporal fusiform cortex, the right temporal pole, the right parahippocampal gyrus, the inferior temporal gyrus and the left insular cortex (**Table 6.2.** and **Figure 6.1.**). The temporal fusiform cortex and temporal pole have been reported to show cortical thickness differences over time between UHR-T and controls but not between UHR-NT and controls (Ziermans, Schothorst et al. 2012). The temporal pole is thought to be implicated in different cognitive functions such as emotion, attention, behaviour, and memory (Blaizot, Mansilla et al. 2010). In people with schizophrenia, abnormalities in this region have been associated with a range of clinical symptoms including, amongst others, auditory hallucinations and thought disorder (Barta, Pearlson et al. 1990, Shenton, Kikinis et al. 1992). The temporal fusiform cortex plays a central role in facial configuration processing in the healthy brain (LaBar, Crupain et al. 2003). Deficits in this domain have been recently reported in the UHR population, and may be one of the factors that underlie social dysfunction in schizophrenia (Kim, Shin et al. 2010). The parahippocampal gyrus has also been reported to show reduced thickness both in the UHR (Jung, Kim et al. 2011, Tognin, Riecher-Rossler et al. 2013) and first episode psychosis (Schultz, Koch et al. 2010). Specifically, this area has been identified as a site of robust structural and functional alteration in UHR individuals (Allen, Seal et al. 2011) and those who have developed the disorder (Shenton, Dickey et al. 2001, Seidman, Pantelis et al. 2003). The right inferior temporal gyrus volume has also been reported as progressively reduced overtime in UHR-T compared to UHR-NT (Borgwardt, McGuire et al. 2008). Finally the left insular cortex plays a key role in emotional regulation, which is typically altered in psychosis, and has been found to show reduced volume in UHR-T compared to UHR-NT (Borgwardt, McGuire et al. 2008, Takahashi, Wood et al. 2009).

While the results of the present investigation provide further evidence for the implication of the above regions in schizophrenia, it should be noted that in multivariate methods an individual region may display

high discriminative power due to two possible reasons: (i) a difference in volume between groups in that region; (ii) a difference in the correlation between that region and other areas between groups. Thus, the regions identified in this work should be interpreted as parts of a spatially distributed pattern rather than as independent areas. In addition, it should be noted that these regions were identified using an arbitrary threshold of 30% based on previous studies (Gong, Wu et al. 2011, Gong, Li et al. 2013), and that prediction of symptom progression was to some extent informed by all voxels in the brain since no feature extraction was employed.

In contrast to the results obtained using RVR, the univariate analysis of the structural MRI data, in which each voxel is considered as a spatially independent unit, did not detect any regions that showed a significant association with progression of symptoms after correction for multiple comparisons. This supports the idea that multivariate methods such as RVR are more sensitive to the subtle and spatially diffuse alterations typically observed in psychiatric disorders, and therefore may be better suited to the possible development of clinical diagnostic tools, than standard mass-univariate techniques (Brammer 2009).

The present work has four main limitations. Firstly, the number of subjects included in the study was relatively small and therefore the generalizability of the results is unclear. Multi-centre studies would be needed in order to better characterize the predictive value of structural neuroimaging for predicting symptom progression in real-life clinical practice. Secondly, 30% of the participants had been exposed to antipsychotic medication which might have influenced the present results for instance by resulting in changes in brain structure while also influencing symptom progression. Nevertheless, as reported in the Results section of this chapter, there is no evidence for an association between antipsychotic medication at first clinical presentation (i.e. yes/no) and progression of illness. Thirdly, there are a number of

potential sources of individual variability in symptom progression that were not included in the statistical model; these include, for example, sociodemographic variables such as age, gender and ethnicity, and treatment course variables such as life events and psychosocial interventions during the follow-up period. It is expected that the integration of this information within the same statistical model would improve prediction of symptom progression. Fourthly, in the present investigation I examined the predictive value of gray rather than white matter as the former could be estimated more accurately than the latter. However, given the number of studies reporting an association between white matter integrity and clinical outcome in the UHR population (Walterfang, McGuire et al. 2008, Karlsgodt, Niendam et al. 2009, Bloemen, de Koning et al. 2010, Carletti, Woolley et al. 2012, Ziermans, Schothorst et al. 2012), it would be interesting to use DTI scans in future studies.

In conclusion, the results of the present study provide proof-of-concept that it might be possible to use structural neuroimaging to inform quantitative prediction of subsequent progression of symptoms in individuals at UHR for psychosis. This would enable clinicians to target those individuals at greatest need of preventative interventions thereby resulting in a more efficient use of health care resources. It should be noted, however, that daily clinical practice often requires clinicians to make prompt treatment decisions, and delaying the decisional process in order to acquire and analyse structural neuroimaging data could be impractical and potentially harmful to a patient. A possible solution would be the development of a practical and flexible analytical tool for clinical use that does not require the manual implementation of a lengthy pipeline. In addition, it is likely that the use of structural neuroimaging in everyday clinical practice would ultimately require a greater accuracy of prediction than that found in the present study. Such accuracy might be improved, for example, by combining structural neuroimaging with other types of data, an integrative approach which

was successfully applied to an investigation of mild cognitive impairment (Fan, Resnick et al. 2008).

7. Conclusions

7.1. Summary of hypotheses and main findings

The main aim of the present doctoral work was to (i) investigate neuroanatomical abnormalities in individuals at ultra high risk for psychosis and (ii) compare those individuals who subsequently made transition to psychosis with those who did not using a sample that is larger and more representative of the general population compared to previous studies. I therefore combined structural magnetic resonance imaging data collected at four different research centres: London, United Kingdom; Basel, Switzerland; Melbourne, Australia; and Munich, Germany. Each centre provided imaging data in an UHR group and a group of healthy controls. At each site UHR individuals were scanned at first clinical presentation and were then followed up for at least two years to allow further sub-categorisation according to clinical outcome (i.e. UHR-T and UHR-NT sub groups). The MRI data collected at the different sites were combined to form groups of UHR-T, UHR-NT and healthy control subjects larger than any previous UHR neuroimaging study. I analysed the MRI structural data using different analytic techniques: two univariate techniques, voxel-based morphometry (VBM), voxel based cortical thickness (VBCT), and two multivariate machine learning analytic techniques, Support Vector Machines (SVM) and Relevance Vector Regression (RVR).

My first objective was to use VBM and VBCT to investigate whether there are volumetric and cortical thickness abnormalities that distinguish UHR individuals from healthy controls at the group level. This first objective led me to test the following two hypotheses:

Hypothesis 1, Chapter 3

My first hypothesis was that UHR individuals would show volumetric abnormalities relative to healthy controls, and that these would be qualitatively similar to those observed in patients with established

schizophrenia (i.e. volumetric reductions). This first hypothesis was confirmed as the UHR group showed significant volumetric reductions in the prefrontal and anterior cingulate regions, areas that have been previously reported to be affected in patients with first episode and established psychosis (Steen, Mull et al. 2006, Olabi, Ellison-Wright et al. 2011, Vita, De Peri et al. 2012).

Hypothesis 2, Chapter 4

My second hypothesis was that the UHR group would show cortical thickness abnormalities relative to healthy controls in areas that have been reported in previous studies of cortical thickness in UHR subjects and patients with a first episode of psychosis such as the frontal, anterior cingulate, parahippocampal, temporal, parietal cortices and the precuneus (Narr, Bilder et al. 2005, Fornito, Yucel et al. 2008, Fornito, Yung et al. 2008, Schultz, Koch et al. 2010, Ziermans, Schothorst et al. 2012). A mask, including these regions of interest (ROIs), was employed in the analysis of cortical thickness. This hypothesis was in part confirmed as the UHR group showed cortical thinning of the right parahippocampal cortex compared to the healthy controls group. At a less conservative threshold ($p < 0.001$ uncorrected), a trend for cortical thinning in the inferior part of the left parahippocampal gyrus was also detected.

My second objective was to use VBM and VBCT to investigate whether there are volumetric and cortical abnormalities that can help distinguish between UHR individuals who subsequently made transition to psychosis and those who did not at the group level. This second objective led me to test the following two hypotheses:

Hypothesis 3, Chapter 3

My third hypothesis was that individuals at UHR who subsequently made transition to psychosis would show differences in gray matter volume relative to those who did not become ill at presentation. Areas

identified in previous neuroimaging studies of first episode and established psychosis, such as the inferior frontal, parahippocampal and superior temporal regions (Job, Whalley et al. 2003, Farrow, Whitford et al. 2005, Job, Whalley et al. 2005) were used as regions of interest. A mask, including these ROIs, was employed in the analysis of gray matter volume. This hypothesis was in part confirmed as the UHR-T group showed relatively reduced gray matter volume in the anterior part of the left parahippocampal gyrus, bordering with the hippocampal uncus compared to UHR-NT.

Hypothesis 4, Chapter 4

My forth hypothesis was that the UHR-T group would show more pronounced cortical thickness abnormalities in the frontal, anterior cingulate, parahippocampal, temporal, parietal cortices and the precuneus areas than UHR-NT (Narr, Bilder et al. 2005, Fornito, Yucel et al. 2008, Fornito, Yung et al. 2008, Schultz, Koch et al. 2010, Ziermans, Schothorst et al. 2012). A mask, including these ROIs, was therefore employed in the analysis of cortical thickness. This hypothesis was not confirmed, as there were no cortical thickness differences between the UHR-T and UHR-NT that survived correction for multiple comparisons. However, at a less conservative threshold ($p < 0.001$ uncorrected), there was a trend for cortical thinning in the orbital part of the left inferior frontal gyrus in the UHR-T group compared to the UHR-NT.

My third objective was to use a multivariate pattern classification approach, such as Support Vector Machines (SVMs), to investigate whether it is possible to distinguish between UHR and healthy controls at the individual level using structural imaging data. Therefore I used the images pre-processed for the first two studies described in Chapters 3 and 4 to investigate whether gray matter volume and cortical thickness information would allow an accurate and significant classification of UHR and healthy controls. This third objective led me to test the following hypothesis:

Hypothesis 5, Chapter 5

My fifth hypothesis was that gray matter volume and cortical thickness would allow an accurate classification discerning UHR individuals from healthy controls. This hypothesis was in part confirmed as the application of SVM allowed UHR to be successfully distinguished from healthy controls at the individual level based on gray matter volume information (balanced accuracy 56.8%, p = 0.0310). On the contrary, cortical thickness information did not allow accurate discrimination of UHR from healthy controls. The gray matter regions that held the highest discriminative values were distributed in a relatively widespread, bilateral network that include the inferior, superior, and middle frontal gyrus, middle temporal gyrus, lingual gyrus, precuneus and cuneus, inferior parietal lobule and angular gyrus. The application of SVM to gray matter volume further allowed an accurate discrimination of UHR-NT from healthy controls (balanced accuracy = 59.8%, p = 0.0050). The areas that held the highest discriminative values were distributed in a bilateral network of regions, including the middle frontal gyrus, lingual gyrus, middle occipital gyrus, cuneus, precentral and postcentral gyrus, angular gyrus, middle and superior temporal gyrus and sub-gyral region. In contrast, gray matter volume did not allow an accurate discrimination of UHR-T individuals from healthy controls. Cortical thickness did not allow the discrimination of either UHR-T or UHR-NT from healthy controls.

My forth objective use SVM to distinguish between UHR-T and UHR-NT using gray matter volume and cortical thickness information, as this would be the most clinically useful application. I used the images pre-processed for the VBM and VBCT analyses to investigate whether gray matter volume and cortical thickness would allow an accurate and significant classification of UHR-T and UHR-NT at the individual level. This fourth objective led me to test the following hypothesis:

Hypothesis 6, Chapter 5

My sixth hypothesis was that gray matter volume and cortical thickness would allow an accurate classification discerning UHR-T from UHR-NT. This hypothesis was in part confirmed as the application of SVM allowed UHR-T to be successfully distinguished from UHR-NT based on cortical thickness information (balanced accuracy 56.5%, p = 0.0470). The regions that held the highest discriminative values were distributed in a relatively widespread bilateral network of regions that includes the middle temporal gyrus, subcallosal gyrus, medial and inferior frontal gyrus, lingual gyrus, parahippocampal gyrus, orbital gyrus and fusiform gyrus. In contrast, gray matter volume did not allowed significant discrimination.

My fifth objective was to use a multivariate classification approach to predict symptom progression at the individual level in UHR individuals using structural imaging data. This analysis was performed in a subsample of UHR individuals recruited at the London site for which baseline and two years follow-up information of clinical scores (i.e. PANSS) were available. This fifth objective led me to test the following hypothesis:

Hypothesis 7, Chapter 6

I hypothesized that gray matter volume and cortical thickness respectively, would allow quantitative prediction of symptom progression at the individual level with statistically significant accuracy. This hypothesis was in part confirmed as cortical thickness information allowed accurate prediction of symptom progression at individual level (correlation = 0.34, p = 0.026, Mean Squared-Error = 249.63, p = 0.024). The cortical regions that held the highest weight vector score included the left insular cortex and lateral and medial regions of the right temporal cortex. In contrast, the application of RVR to gray matter volume information did not allow accurate prediction of symptom progression.

7.2. Discussion

7.2.1. Implications for the neurobiological models of psychosis

The neurodevelopmental model of psychosis postulate that the onset of the illness is the end stage of an abnormal neurodevelopmental process that started several years earlier (Weinberger 1987, Murray 1994, Rapoport, Giedd et al. 2012). In the last two decades, neuroimaging studies conducted in young individuals at ultra high risk for psychosis, first episode of psychosis and relatives of patients with established schizophrenia, have provided strong evidence that brain abnormalities associated with schizophrenia are indeed already present before the illness onset. These brain abnormalities are thought to result from early brain insults that can arise as early as the pre or perinatal period (Weinberger 1987, Murray 1994, Rapoport, Giedd et al. 2012). While evidence from neuroimaging studies in early psychosis and high-risk cohorts is largely consistent with the neurodevelopmental model of psychosis, the delay between the early brain insults and the onset of the symptoms remained poorly understood. A possible explanation that has been proposed is that behavioural abnormalities resulting from non-progressive early brain insults would become evident in adolescence/early adulthood, when there is an acceleration of normal brain maturation and the brain circuits are placed under higher functional demand (Weinberger 1987). Throughout the years, the original neurodevelopmental model has been expanded to include new findings that may help explain the delay in the manifestation of the psychotic symptoms. For example, several studies have provided evidence for the mediating effect of environmental risk factors or social stressors (Corcoran, Walker et al. 2003), such as urbanicity (Thornicroft, Bisoffi et al. 1993, March, Hatch et al. 2008, van Os, Kenis et al. 2010), childhood trauma and abuse (Morgan and Fisher 2007, Arseneault, Cannon et al. 2011), ethnic minority status (Bourque, van der Ven et al. 2011), and social adversity (Wicks, Hjern et al. 2005). Although the pathophysiology of the disorder remains poorly

understood, the mechanisms underlining the abnormal neurodevelopment are likely to result from an interaction between early brain insults and environment-dependent dynamic changes such as an excessive elimination of synapses as well as loss of plasticity during adolescence (Keshavan 1999, Keshavan and Hogarty 1999). Findings from neuroimaging studies have also suggested that brain abnormalities that are presents before the disorder becomes frank, become more pronounced around the time of transition to psychosis (Steen, Mull et al. 2006). Thus, the brain abnormalities that are typically observed in chronic schizophrenia result from a combination of neural deficits that are expressed during brain development, neural deficits that become evident around the time of the first psychotic episode and alterations associated with psychotropic medication (Keshavan 1999, Keshavan and Hogarty 1999, Pantelis, Yucel et al. 2003, Ho, Andreasen et al. 2011).

Over the past two decades, neuroimaging studies have provided a rich body of evidence in support of the neurodevelopmental model. In particular, studies of the UHR population have demonstrated that there is evidence for the presence of early brain abnormalities before the onset of full-blown psychosis and that the areas most affected are fronto-temporal regions, similar to abnormalities reported in first episode psychosis and established schizophrenia cohorts (Steen, Mull et al. 2006, Olabi, Ellison-Wright et al. 2011, Vita, De Peri et al. 2012).

Univariate analyses

Consistent with the neurodevelopmental model of psychosis, this doctoral work provides evidence for the existence of neuroanatomical abnormalities in the UHR population compared to healthy controls. Volumetric reductions were observed in the UHR group in the frontal regions bilaterally, in areas previously reported to be affected in patients with first episode and established psychosis (Steen, Mull et al. 2006, Olabi, Ellison-Wright et al. 2011, Vita, De Peri et al. 2012).

Additionally, VBCT analysis revealed cortical thinning in the right parahippocampal gyrus, and at a less conservative threshold ($p < 0.001$ uncorrected) in the left parahippocampal gyrus, in UHR compared to healthy control subjects. These observations are consistent with previous structural imaging studies that have reported gray matter abnormalities in frontal, temporal, and midline limbic structures in high risk individuals, first episode and established schizophrenia (Job, Whalley et al. 2003, Steen, Mull et al. 2006, Jung, Kim et al. 2011, Olabi, Ellison-Wright et al. 2011, Vita, De Peri et al. 2012). The fact that both volumetric and cortical structural alterations were also present in individuals that did not develop psychosis, suggests that these may represent neurobiological markers of vulnerability to psychosis rather than markers of the disease emergence. Another possibility is that some of the individuals that have not made transition to psychosis at the time of the last follow-up within the research study will make transition at a later time or they might develop or have already developed another Axis I disorder. The structural abnormalities detected in this sample appear to support the neurodevelopmental model of psychosis, which states that neuroanatomical abnormalities are present before the onset of the disorder. These abnormalities might reflect neurodevelopmental lesions that occur in the peri-natal phase; however, they could also be the result of an interaction between early insults of the brain and dynamic processes undergoing during adolescence and early adulthood.

As part of this doctoral work I also examined whether the analyses of gray matter volume and cortical thickness would have provided complementary information about neuroanatomical differences between people at high risk for psychosis and healthy controls. VBM and VBCT identified structural abnormalities in UHR compared to controls in different locations of the brain. The fact that VBM and VBCT identified different frontal and temporal abnormalities appears to suggest that these are the result of distinct pathological and neurobiological mechanisms. Consistent with this, recent evidence

suggests that cortical surface and thickness are relatively independent from a genetic and phenotypic perspective, and that the local cortical volume is more closely related to surface area (i.e. gyrification) than to cortical thickness (Winkler, Kochunov et al. 2010). Thus, the frontal abnormalities observed in the UHR population could be more associated to sulcal and gyral anomalies while temporal abnormalities could depend more on cortical atrophy. We know that sulcal and gyral folding is almost complete during the third trimester of gestation (Chi, Dooling et al. 1977, Worthen, Gilbertson et al. 1986) and remains relatively stable after birth (Zilles, Armstrong et al. 1988, Armstrong, Schleicher et al. 1995), consequently the volumetric reductions observed in the frontal regions are more likely to reflect early neurodevelopmental abnormalities. In contrast, cortical thinning of the right parahippocampal region observed in the UHR population could could be the result of early brain insults that occurred during brain development or, alternatively, neurodevelopmental changes taking place around adolescence/early adulthood.

The neurodevelopmental model also supports the idea of the presence of more marked structural abnormalities in those UHR who will go on to develop psychosis compared to those who will not. This doctoral work demonstrated that volumetric reductions are present in UHR-T individuals in the anterior part of the left parahippocampal gyrus, bordering with the hippocampal uncus compared to UHR-NT. The analysis of cortical thickness did not provide further evidence, as there were no cortical thickness differences between the UHR-T and UHR-NT that survived correction for multiple comparisons. The fact that VBM but not VBCT was able to detect neuroanatomical abnormalities in the comparisons between UHR-T and UHR-NT groups suggests that alterations observed in this area of the left parahippocampal gyrus are likely to reflect abnormalities in sulcal and gyral folding and therefore arising early in life in the pre-natal phase constituting a marker of vulnerability rather than a marker of the emergence of the disease.

The structural abnormalities observed in the fronto-temporal regions are also in line with studies that focussed on healthy individuals with high scores in psychometric schizotypy (Ettinger, Williams et al. 2012, DeRosse, Nitzburg et al. 2015), further supporting the idea of a phenomenological and phonotypical continuum with schizophrenia spectrum disorders.

Taken together, these findings support the idea that the abnormalities observed in UHR for psychosis represent an interconnected network of fronto-temporal regions rather than being isolated abnormal areas in the brain (Insausti, Amaral et al. 1987, Cavada, Company et al. 2000).

Multivariate analyses

Previous neuroimaging studies of UHR for psychosis suggest that the neuroanatomical abnormalities observed in this population are neither necessarily focal nor spread in the entire brain but seem to be involved in an interconnected network of fronto-temporal regions. Multivariate machine learning approaches that take into account the inter-relationship between different measures (e.g. gray matter volume across different voxels) are better suited to detect subtle and spatially distributed patterns of network alterations. Therefore, I employed a multivariate machine learning technique, Support Vector Machine (SVM), to investigate whether volumetric or cortical network information would allow an accurate prediction of group membership at the individual level.

The hypotheses were partially confirmed as volumetric information allowed accurate classification of group membership for UHR and healthy controls, whilst cortical thickness information allowed an accurate prediction of group membership of UHR-T and UHR-NT. Interestingly, the frontal regions that showed significant volumetric reductions in the univariate analyses of gray matter volume (i.e. VBM)

are also contributing to the accurate classification in the multivariate analysis. Conversely, cortical thickness data did not allow accurate discrimination between UHR and healthy controls. On the other hand, the application of SVM to cortical thickness information rather than volume, did allowed the successful discrimination of UHR-T and UHR-NT. Interestingly, the parahippocampal cortical thickness, identified by the univariate analysis, was among the areas that were determinant in the discrimination. The VBCT analysis revealed that there were no cortical thickness differences between the UHR-T and UHR-NT that survived correction for multiple comparisons. However, using a less conservative threshold ($p < 0.001$ uncorrected) there was a trend for cortical thinning in the UHR-T group compared to the UHR-NT in the orbital part of the left inferior frontal gyrus, area that also appear to be determinant in the multivariate classification. The fact that cortical thickness information but not volumetric information allowed successful classification of UHR-T and UHR-NT at the individual level might suggest that cortical thickness abnormalities in UHR-T are more subtle and widespread rather than focal, this could in part explain why these were not detected by the univariate analysis of cortical thickness. On the other hand, volumetric difference may be more pronounced but also more focal consistent with findings from the VBM study reported in Chapter 3. These findings suggest that network abnormalities in gray matter volume could be more associated to vulnerability to psychosis or alternatively the UHR stage within the staging model (McGorry, Keshavan et al. 2014), whilst cortical network abnormalities could be more associated to transition to psychosis. The results of this work provide further evidence for the implication of the above regions in psychosis; however, as discussed in Chapter 5, the regions identified in the present study should be interpreted as parts of a spatially distributed pattern of neuroanatomical alterations rather than as independent areas.

A recent follow-up study on the outcome of young people initially identified as being at ultra high risk for psychosis, showed that only 30% of those who do not make transition to psychosis reach full symptomatic and functional recovery, 26% reach symptom remission without functional recovery and 26% meet criteria for functional recovery without symptom remission (Schlosser, Jacobson et al. 2012). Another follow-up study reported that 25% of these individuals still present symptoms that qualify them as being at risk for psychosis (Velthorst, Nieman et al. 2011). This provides a rationale for investigating not only predictors of transition to psychosis but also predictors of symptom progression above and beyond psychosis outcome. I have therefore applied a multivariate machine learning method, RVR to investigate whether structural information would have allowed the quantitative prediction of symptom progression (i.e. PANSS total scores) at the individual level in a subsample of UHR recruited at the London site. The application of RVR revealed that cortical thickness but not volumetric information allowed the quantitative prediction of symptom progression in UHR individuals above and beyond transition to psychosis. This provides preliminary evidence that symptom progression in UHR people is associated with differences in cortical thickness rather than volumetric abnormalities.

7.2.2. Clinical implications

The possibility of elucidating neuroanatomical abnormalities in the early stages of psychosis has important implications both for the understanding of pathophysiological mechanisms underlining this disorder and for the identification of neuromarkers that can be used to inform clinical practice. To date, all the UHR individuals entering the early intervention pathways are offered the same therapeutic options as it is not possible to predict which of them will go on to develop psychosis only based on the clinical presentation (Nelson 2010). Neuroimaging studies of UHR hold the potential of elucidating the mechanisms underlining psychosis onset and at the same time of

informing the early detection and the diagnosis of transition to psychosis. The present multi-centre investigation revealed that there are volumetric and cortical differences between UHR individuals and healthy controls and volumetric differences between UHR-T and UHR-NT. Although the observed volumetric and cortical differences contribute to the understanding of the pathophysiology of the disease, their clinical applicability is unclear as these results are statistically significant only at the group level.

Neuroimaging, that allows the study of the brain in vivo, holds the promise of elucidating the pathophysiological mechanisms underling the emergence of psychosis and providing information that can help to predict psychosis outcome. This would potentially allow a more targeted and ethically safer approach to the treatment of individuals at ultra high risk of psychosis. Multivariate machine learning techniques can employ information derived from any kind of multivariate data, including brain scans, to potentially predict the psychosis outcome at the individual level. Although the results from the present doctoral work were statistically significant, accuracy remains relatively low to be usefully applied in real-world clinical settings. For example, the accuracies obtained in the present work are below the 90% mark that was suggested for routine clinical use of MRI (Kasparek, Thomaz et al. 2011, Iwabuchi, Liddle et al. 2013). Furthermore, recent studies have emphasized the need to report additional measures such as Diagnostic Odd Ratio (DOR), which provides a mean for synthesising SVM-based findings in a meta-analytical framework, and Number Needed to Predict (NNP), that provides a summary value for measuring economic effects and resources allocation required to deploy SVM-based diagnostic tools at a service level (Iwabuchi, Liddle et al. 2013). The use of these alternative measures may be particularly useful given that different studies investigating the same populations have reported different levels of accuracy (Orru, Pettersson-Yeo et al. 2012). In order to facilitate the application of these approaches in clinical settings it would

be therefore essential to report measures that empathise the clinical significance of the results (Iwabuchi, Liddle et al. 2013). During the last two decades neuroimaging studies have provided a large amount of data that up till now failed to have a real clinical impact. In order to evaluate the clinical utility of a potential biomarker or diagnostic/prognostic approach, several factors should be considered. In particular, the relevance of the outcome and the number of patients that needs to be assessed in order to make one successful prediction have to be balanced against potential risks and side effects, burden and delays associated to the marker testing, costs and ethical issues (Perlis, Patrick et al. 2009, Perlis 2011, Prata, Mechelli et al. 2014).

The UHR population is clinically more heterogeneous than first episodes or schizophrenia populations (Fusar-Poli, Bechdolf et al. 2013). Indeed, the majority of the individuals does not make transition to psychosis, and those who do become ill can develop schizophrenia, affective or other psychotic disorders (Fusar-Poli, Bechdolf et al. 2013). Among those individuals that will not develop psychosis, about the 25% will not reach full symptomatic remission after three years (Velthorst, Nieman et al. 2011) and among those who reach full or partial remission of positive symptoms a portion will still present with relatively low social and occupational functioning (Addington, Cornblatt et al. 2011). This partially shifts the focus from prediction of outcome to prediction of symptom progression and functioning over time. While preventing transition to psychosis remains a critical goal of early intervention services, there is growing appreciation that some individuals who never develop psychosis show disabling symptoms and poor functioning that have a detrimental impact on their quality of life. Neuroimaging and machine learning applications hold the potential of predicting functional outcome and symptom progression overtime (Allen, Chaddock et al. 2014). Results form the present work demonstrated that cortical thickness information allows quantitative prediction of symptom progression in UHR for psychosis regardless of

the psychosis outcome. This application of machine learning would potentially inform clinical practice and the selective delivery of early interventions, for example by providing further support to those individuals more likely to present with functional impairment and disabling symptoms overtime. However, this analysis was performed in a relatively small subsample of UHR for psychosis, replication of this finding in a larger, independent sample is required to draw more definitive conclusions.

7.3. Strengths

The work presented here has a number of strengths. Firstly, it employed a multi-centre approach for the investigation of structural abnormalities in individuals at high risk for psychosis. Relative to a single-centre study, this approach has several advantages. Firstly, it increases sample size by pooling MRI data collected at different sites; this is particularly important for analyses of clinical outcome (i.e. statistical comparisons between UHR-T and UHR-NT subjects) as UHR-T samples sizes tend to be small at a single site level. In addition to the increased statistical power and the reduced probability of type I errors (Button, Ioannidis et al. 2013), combining data from different centres has the advantage of enhancing the generalizability of the results. Data collected from single research centres may differ due to variability in genetic, socio-demographic and environmental factors; in contrast, the results presented in this doctoral work are based on data collected in the UK, Germany, Switzerland and Australia and therefore should have greater generalizability.

Secondly, the sample reported here are individuals who were at high risk of developing a psychotic illness but still considered not psychotic. The study of the high risk population offers a window in which the mechanisms underling the emergence of psychosis can be studied with a relatively limited impact of confounding factors such as chronicity and psychotropic medications.

Thirdly, different analytic techniques have been used to investigate the pathophysiology of the illness. Specifically, in order to examine gray matter volume and cortical thickness, two voxel-based analytic techniques have been employed: VBM and VBCT. Both techniques allow the investigation of volume and cortical thickness abnormalities at the voxel level. Previous studies investigating cortical thickness in the UHR, first episode and established schizophrenia have used surface-based analytical techniques, which complicates the direct comparison of regional volumetric, or thickness abnormalities. VBM and VBCT use the same spatial preprocessing methods therefore it is possible to compare the patterns of alterations in gray matter volume and cortical thickness, minimising possible methodological differences.

Fourthly, multivariate machine learning techniques, including SVM and RVR, were applied to a relatively large sample of UHR. Multivariate machine learning techniques allow inferences at the level of the individual, and therefore have greater translational potential in everyday clinical practice than standard analytical approaches based on group-level statistics.

Fifthly, in this doctoral work I examined for the first time whether it is possible to use neuroanatomical information to make accurate quantitative predictions of symptom progression in individuals at UHR for psychosis. Although the analysis was performed only on a subsample of UHR, for which the clinical information were available at baseline and two-years follow-up, the results provide proof-of-concept that it might be possible to use brain structural information to inform quantitative prediction of symptom progression in the population at high risk for psychosis.

7.4. Limitations

A number of limitations also need to be considered when interpreting the results reported in this thesis. Firstly, the structural MRI data were

collected using different scanners and acquisition sequences**.** Although this has been taken into account when the statistical analyses were preformed and this method has been previously successfully employed (Stonnington, Tan et al. 2008, Segall, Turner et al. 2009, Suckling, Barnes et al. 2010), it cannot be completely ruled out that this might have had an impact on the findings. In particular, although it was possible to control for scanner effects in the VBM and VBCT analyses by modelling scanner as a factor in the statistical analyses, this was not possible in the SVM analyses. Therefore, the use of different scanners and acquisition sequences is most likely to have added noise to the data, increasing the unexplained variance in the statistical analysis and lowering the probability of finding statistically significant results.

Secondly, although the four centres employed comparable inclusion criteria, different screening instruments and selection procedures were used to identify individuals at ultra high risk for psychosis. A recent meta-analysis reported that different screening instruments are associated with different transition rates (Fusar-Poli, Bonoldi et al. 2012). In addition, variability could in part be due to different sampling selections procedures at the recruiting centres. When exploring between-centre sociodemographic characteristics, a number of differences were detected. In particular, differences in age, ethnicity and total gray matter volume were observed between centres when comparing each sub-sample (i.e. HC, UHR-T and UHR-NT) (Table A3.1. and A3.2. Appendix 3). As discussed in previous chapters, these features were modelled as covariates of no interest when performing the statistical analyses in order to reduce their impact on the findings; however, it is not possible to exclude that the observed differences might have contributed noise to the data which, in turn, reduced the chance of finding reliable differences in brain structure. In addition, although nominally different transition rates were observed in the different centres, there was no significant effect of site on the percentage of those who transitioned to psychosis. This

sociodemographic variability could be minimised in future multi-centre studies by employing the same inclusion criteria, screening instruments and selection procedures at each site.

Thirdly, the data analysed in the present doctoral work were collected at a single cross-sectional time point. Therefore, it was not possible to assess the stage at which the observed differences had emerged. This issue could be addressed by using a longitudinal approach where individuals at high risk for psychosis are scanned and assessed at different time points not only in the prodromal phase but also after the onset of psychosis.

Fourthly, the UHR population is heterogeneous in at least two ways. Firstly, UHR individuals can meet different inclusion criteria. The criteria require at least one of the following presentations: "attenuated" psychotic symptoms, brief limited intermittent psychotic symptoms (BLIPS) lasting less than a week and resolving spontaneously without the use of antipsychotic medication, or a significant decrease in functioning in the context of genetic risk for psychosis (Yung, Yuen et al. 2005). Therefore UHR included in this and previous studies can have rather different clinical presentations, and they can follow different trajectories in terms of psychosis outcome, symptoms remission and functional recovery (Velthorst, Nieman et al. 2011, Addington and Heinssen 2012, Schlosser, Jacobson et al. 2012, Fusar-Poli, Bechdolf et al. 2013). Secondly, UHR individuals can present with different comorbidities, the most common being anxiety, depression, substance misuse and personality disorders (Woods, Addington et al. 2009) that can further complicate the picture. Recent studies reported that about 69% of the UHR population had one or more mood/anxiety diagnoses, 25% had substance abuse or dependence, 44% had one or more Axis II diagnoses. Another recent study reported that 73% of UHR had a comorbid Axis I diagnosis and 40% of the cohort had a comorbid depressive disorder (Fusar-Poli, Nelson et al. 2014). The presence of

Axis I or II comorbidities associated to the UHR state can correspond to potentially different trajectories in terms of outcome and therefore potentially different underlying psychophysiological mechanisms (Modinos, Allen et al. 2014). Such heterogeneity within the UHR group was not modelled in the statistical analyses and may have reduced statistical sensitivity to neuroanatomical abnormalities.

Fifthly, within-centre analyses are not reported in the present doctoral work. I did not attempt to replicate within-centre analyses (e.g.(Pantelis, Velakoulis et al. 2003, Borgwardt, McGuire et al. 2007, Borgwardt, McGuire et al. 2008, Fornito, Yung et al. 2008, Meisenzahl, Koutsouleris et al. 2008, Koutsouleris, Meisenzahl et al. 2009, Koutsouleris, Schmitt et al. 2009) as this was not part of the objectives of the present doctoral work. This is because (i) different software (e.g. PRoNTo *versus* in-house script used by Koutsouleris et al. 2008) and different analytic methods (e.g. VBCT *versus* Freesurfer used by Fornito et al 2008) were used in this work compared to other previously published studies; and (ii) the samples included in the present work overlapped but did not exactly match with those used in previous papers (e.g. (Pantelis, Velakoulis et al. 2003, Fornito, Yung et al. 2008, Koutsouleris, Schmitt et al. 2009).

7.5. Future developments

The present doctoral work provides further evidence supporting the feasibility and utility of using a multi-centre approach when studying clinical populations that are difficult to recruit (e.g. the UHR population) and where the phenomenon of interest is rather infrequent (e.g. transition to psychosis is estimated to occur in around 29% of those who meet criteria for UHR within 24 months although at some centres this transition rate may be even lower). To overcome limitations associated with cross-sectional and prospective studies reported here, future multi-centre studies should employ a longitudinal rather than cross-sectional design; this would allow a better temporal

characterization of the emergence of neuroanatomical abnormalities and how these trajectories varies depending on clinical outcome. Individuals at UHR for psychosis could be further stratified according to the inclusion criteria and symptomatology at baseline to investigate whether different subgroups are associated with distinct underling mechanisms. Moreover a retrospective stratification of UHR-T according to different diagnosis at follow-up such as schizophrenia, other psychosis, or affective disorders would allow one to further characterise the neuropathological pathways underlying the different illnesses. In addition, future studies would benefit from using the same scanning sequences. On this regard, the ADNI consortium has developed a structural MRI sequence that generates an image with similar properties independently of the scanner model and manufacturer (Jack, Bernstein et al. 2008). Currently, there are multi-centre projects such as EU-GEI (European network of national schizophrenia networks studying gene-environment interaction) (European Network of National Networks studying Gene-Environment Interactions in, van Os et al. 2014) and NAPLS (North American Prodrome Longitudinal Study), (Cannon, Cadenhead et al. 2008) that have already adopted this approach, and that will therefore be able to pool different datasets into a single investigation with minimal impact of between-centre differences. In order to promote the translational application of machine learning approaches in real-world clinical practice, future studies should consider developing and testing predictive algorithm in large and independent datasets. In addition, given the inconsistent accuracies reported by studies that compared patients and controls (Orru, Pettersson-Yeo et al. 2012), future studies should aim to report measures that are clinically significant, such as NNP and DOR.

7.6. Conclusions

The present multi-centre investigation of individuals at ultra high risk for psychosis demonstrates that there are volumetric and cortical

differences in fronto-temporal regions associated with vulnerability to psychosis, and that there are temporal volumetric abnormalities in UHR individuals who subsequently develop psychosis relative to those who do not. Using machine learning methods, I also showed that volumetric abnormalities allow the distinction between people at UHR and healthy controls at the level of the individual regardless of transition to psychosis. In contrast, cortical thickness measures appear to better able to classify according to clinical outcome and symptom progression at the level of the individual. However, the classification accuracies obtained from multivariate analyses, although statistically significant, were low and may not be suitable for clinical application.

Taken together these findings suggest that, consistent with the neurodevelopmental model, there are neuroanatomical abnormalities that precede the emergence of psychosis within a distributed fronto-temporal network. The results of the current work show that UHR and healthy controls are distinguishable at the individual level based on information on the gray matter volume, and UHR-T and UHR-NT are distinguishable at the individual level using cortical thickness information. Nevertheless, the accuracies found in the present work remain relatively low to be safely applied in real-word clinical settings. The timing of the emergence of these abnormalities could not be established, and further research is needed to examine whether these have emerged early in life or around adolescence/early adulthood.

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Appendix 1 Publications derived from this thesis

A1.1. Neuroanatomical abnormalities that predate the onset of psychosis

Candidate's contribution to the study: I have carried out the preprocessing and the statistical analyses of the MRI data. I have drafted the first version of the manuscript, I have implemented the suggestions made by the co-authors and I have revised the manuscript according to the reviewers' comments during the publication phase.

ORIGINAL ARTICLE

Neuroanatomical Abnormalities That Predate the Onset of Psychosis

A Multicenter Study

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Context: People experiencing possible prodromal symptoms of psychosis have a very high risk of developing the disorder, but it is not possible to predict, on the basis of their presenting clinical features, which individuals will subsequently become psychotic. Recent neuroimaging studies suggest that there are volumetric differences between individuals at ultra-high risk (UHR) for psychosis who later develop psychotic disorder and those who do not. However, the samples examined to date have been small, and the findings have been inconsistent.

Objective: To assess brain structure in individuals at UHR for psychosis in a larger and more representative sample than in previous studies by combining magnetic resonance imaging data from 5 different scanning sites.

Design: Case-control study.

Setting: Multisite.

Participants: A total of 182 individuals at UHR and 167 healthy controls. Participants were observed clinically for a mean of 2 years. Forty-eight individuals (26.4%) in the UHR group developed psychosis and 134 did not.

Main Outcome Measures: Magnetic resonance images were acquired from each participant. Group differences in gray matter volume were examined using optimized voxel-based morphometry.

Results: The UHR group as a whole had less gray matter volume than did controls in the frontal regions bilaterally. The UHR subgroup who later developed psychosis had less gray matter volume in the left parahippocampal cortex than did the UHR subgroup who did not.

Conclusions: Individuals at high risk for psychosis show alterations in regional gray matter volume regardless of whether they subsequently develop the disorder. In the UHR population, reduced left parahippocampal volume was specifically associated with the later onset of psychosis. Alterations in this region may, thus, be crucial to the expression of illness. Identifying abnormalities that specifically predate the onset of psychosis informs the development of clinical investigations designed to predict which individuals at high risk will subsequently develop the disorder.

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PSYCHOTIC DISORDERS ARE usually preceded by a prodromal phase in which there is a gradual deterioration of global and social functioning and the emergence of attenuated psychotic symptoms.[1,2] However, not all people with these features progress to develop a full-blown psychotic disorder; 20% to 50% develop psychosis, usually within 24 months, but the remainder do not.[3-6] Individuals first seen with this clinical syndrome are, thus, said to be at ultra-high risk (UHR) for psychosis.[7] Results of recent trials[8-10] suggest that clinical intervention in the UHR population may reduce the risk of later transition to psychosis. However, it is difficult on purely clinical grounds to distinguish individuals who will later become psychotic from those who will not.[5,6,10] This prevents the selective provision of potentially preventive interventions to the subgroups most likely to become psychotic, which is desirable from an ethical standpoint and for the efficient use of health care resources.

Recent studies[11-20] using magnetic resonance imaging (MRI) have examined whether there are neuroanatomical differences between UHR individuals who subsequently develop psychosis and those who do not. A variety of differences in regional gray matter volume (GMV) have

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been reported, but the findings have been inconsistent; this may partly reflect the use of small samples. However, UHR individuals are hard to recruit, and it is difficult for any single center to scan a large sample. Sample size is a particular problem for the key comparison between UHR individuals who later develop psychosis and those who do not, which entails a further subdivision of the UHR sample according to clinical outcome. A potential solution is to conduct multicenter studies, with the pooling of data to produce a relatively large total sample. This approach has been successfully used in neuroimaging studies of other central nervous system disorders in which patient recruitment is difficult.[21,22]

In the present study, whole-brain MRIs were acquired from 5 MRI scanners in London, United Kingdom (2 sites); Basel, Switzerland; Munich, Germany; and Melbourne, Australia. The objective was to identify the most robust neuroanatomical abnormalities in individuals at UHR and to compare UHR participants who subsequently made a transition to psychosis with those who did not. At each site, participants were scanned at first clinical presentation and were observed clinically for a mean of 2 years so that they could then be subcategorized according to clinical outcome. The MRI data from each site were combined to form a large UHR sample, which was subdivided into individuals who had developed psychosis (UHR-T) and those who had not (UHR-NT). The MRI data from several matched healthy controls were also acquired at each site.

The first prediction, based on data from previous studies,[11,15,18,23-27] was that the UHR group as a whole would show regional volumetric differences relative to controls that were qualitatively similar to those seen in patients with schizophrenia. We then tested the main hypothesis that UHR-T individuals would show differences in GMV relative to UHR-NT individuals in the inferior frontal, parahippocampal, and superior temporal cortices, the areas most frequently implicated in previous studies. Critically, these predictions were based on the results of previous single-center studies[11,15,18,23-27] that used samples other than those reported in the present multisite investigation.

METHODS

SAMPLE

All the UHR individuals were recruited from specialized clinical services for people at high risk for psychosis. Individuals at UHR scanned at the 2 London sites (Institute of Psychiatry and Maudsley Hospital) were recruited via Outreach and Support in South London (OASIS). Individuals at UHR scanned in Basel were recruited through the clinic for early detection of psychosis (FEPSY) at the Psychiatric Outpatient Department, University Hospital. Those scanned in Munich were recruited through the Early Detection and Intervention Centre for Mental Crisis of the Department of Psychiatry and Psychotherapy, Ludwig-Maximilians-University. The UHR individuals scanned in Melbourne were recruited from the Personal Assessment and Crisis Evaluation (PACE) Clinic. Data were, thus, combined from 5 MRI scanners at the Institute of Psychiatry, the Maudsley Hospital, the University Hospital Basel, the Ludwig-Maximilians-University (Munich), and the Royal Melbourne Hospital. Some of the data from London, Basel, Munich, and Melbourne have been described separately in previous single-center studies.[12-14,17,19,20,28-30] Data were from 182 patients at UHR.

All the UHR individuals at all the sites met the Personal Assessment and Crisis Evaluation criteria for UHR. Inclusion required the presence of 1 or more of the following features: (1) "attenuated" psychotic symptoms, (2) brief limited intermittent psychotic symptoms, or (3) a first-degree relative with a psychotic disorder, plus a marked decline in social or occupational functioning.[2,7] None of the participants met the criteria for additional psychiatric disorders or learning disabilities. Details of the inclusion and exclusion criteria and clinical characteristics of the UHR individuals are reported in eTables 1, 2, 3, 4, 5, 6, and 7 (http://www.archgenpsychiatry.com). Most of the UHR group (168 of 182 individuals [92.3%]) had never taken antipsychotic agents or mood stabilizing drugs, and 14 (7.7%) had been exposed to antipsychotic agents (mean [SD] exposure time, 13.0 [19.3] months).

At each site, controls from the same geographic area as the UHR individuals were recruited through local advertisements. The control sample comprised 167 individuals and was comparable with the total UHR group for sex, age, and race/ethnicity. For all participants, the exclusion criteria were past or present diagnosis of psychiatric illness, previous treatment with antipsychotic drugs, medical illness, family history of psychiatric illness, past or present diseases of the central nervous system, alcohol or other substance abuse or dependence (defined using *DSM-IV* criteria), and pregnancy (eTable 1).

During a mean (SD) of 30.6 (10.4) months of follow-up subsequent to scanning, 48 of the UHR sample (26.4%) developed psychosis (UHR-T) and 134 did not (UHR-NT). Each site yielded a data set that included a UHR-T group, a UHR-NT group, and a control group, except 1 of the London data sets, from which the UHR-T group was too small (n=2) to be included in the combined UHR-T vs UHR-NT comparison (eTable 8).

MRI ACQUISITION

At all 5 sites, volumetric MRIs were acquired using a T1-weighted protocol. At 4 sites, the scanner field strength was 1.5 T, and at 1 site it was 3 T. Three sites used General Electric scanners (Milwaukee, Wisconsin), and 2 used Siemens scanners (Erlangen, Germany). The details of the MRI acquisition sequence varied among scanners (eTable 9).

DATA ANALYSIS

Sociodemographic and Clinical Parameters

Differences in demographics and clinical profile between groups were examined using 1-way analysis of variance for parametric data and a χ^2 test for nonparametric data using a commercially available software program (SPSS, version 17.0 for Windows; SPSS Inc, Chicago, Illinois) (**Table 1**).

Preprocessing

We examined group-related differences in GMV using voxel-based morphometry, as implemented in SPM8 software (http://www.fil.ion.ucl.ac.uk/spm) running under MATLAB 7.1 (The MathWorks, Inc, Natick, Massachusetts). First, T1-weighted volumetric images were preprocessed using the DARTEL (diffeomorphic anatomical registration using exponentiated lie algebra)[31] SPM8 toolbox. This approach involves the creation of a study-specific template and the segmentation of each in-

Table 1. Demographic Data and Global Brain Volumes of Study Samples

Characteristic	Healthy Controls (n = 167)	UHR-T Group (n = 48)	UHR-NT Group (n = 134)	Significance
Age, mean (SD), y	23.5 (4.2)	22.7 (4.5)	23.3 (5.3)	$F_2 = 0.552$ $P = .58$
Sex, No.				$\chi^2_2 = 2.541$
Male	104	15	51	$P = .28$
Female	63	33	83	
Race/ethnicity, No.				$\chi^2_8 = 11.122$
White	140	42	106	$P = .09$
Black	11	1	7	
Asian	10	0	6	
Mixed	6	5	15	
Handedness, No.				$\chi^2_4 = 3.355$
Right	147	44	126	$P = .50$
Left	14	3	6	
Ambidextrous	6	1	2	
GMV, mean (SD), mm^3	0.948 (0.11)	0.945 (0.08)	0.937 (0.10)	$F_2 = 0.450$ $P = .64$

Abbreviations: GMV, gray matter volume; UHR-NT, ultra-high risk without disease transition; UHR-T, ultra-high risk with disease transition.

dividual image using such a template, with the aim of maximizing accuracy and sensitivity.[32] The following steps were followed for voxel-based morphometry preprocessing: (1) checking for scanner artifacts and gross anatomical abnormalities for each subject, (2) setting the image origin to the anterior commissure, (3) using the DARTEL toolbox to produce a high-dimensional normalization protocol,[31] (4) checking for homogeneity across the sample, and (5) using standard smoothing (ie, 8 mm). A "modulation step" was also included in the normalization to preserve the information about the absolute gray matter values.[33,34] After this preprocessing, we obtained smoothed, modulated, normalized data that were used for the statistical analysis.

STATISTICAL ANALYSIS

We performed 2 statistical analyses using SPM8 software. First, an analysis of variance was used to compare gray matter images from UHR individuals (UHR-T and UHR-NT combined) and controls. In this analysis, scanner site was modeled as a factor, resulting in 10 experimental groups. Second, we performed an analysis of variance to compare gray matter images from UHR individuals who later became psychotic (UHR-T), UHR individuals who did not become psychotic (UHR-NT), and controls. In this analysis, scanner site was again modeled as a factor, resulting in 14 experimental groups (1 UHR-T group was too small for analysis [n=2] compared with the UHR-NT group [n=19]; see previously herein). Including scanner site as a factor in the statistical analysis allowed us to (1) model scanner-related variance in the data, which had the effect of reducing error variance and increasing statistical sensitivity, and (2) examine the impact of scanner site by testing for scanner effects and scanner × group interactions. To assess how much of the interindividual variance in regions that differed between UHR-T and UHR-NT was explained by diagnostic group and scanner site, respectively, we used the η_p^2 measure of effect size in SPSS. In both analyses, we modeled age, sex, race/ethnicity, and use of medication as covariates of no interest to reduce the potential impact of these variables on the findings. To identify regionally specific changes that were not confounded by global differences, we used the proportional scaling option. Statistical inferences were made at $P < .05$ after familywise error (FWE) correction, with an extent threshold of 5 voxels.

REGION-OF-INTEREST ANALYSES

In addition to the whole-brain analysis, regions of interest (ROIs) were used to examine between-group differences in areas in which volumetric abnormalities have previously been identified in studies of people at high risk for psychosis or patients with first-episode psychosis.[16,33,36] These ROIs were the left parahippocampal gyrus (MNI [Montreal Neurological Institute] coordinates x, y, z, –23, 6, and –20),[35] the right inferior frontal gyrus (45, 37, and 0),[36] and the left superior temporal gyrus (–49, –31, and 2).[16] We used the coordinates from the previous studies that had reported the most significant effects in these regions; none of these previous studies had included data from individuals who participated in the present investigation. Using the SimpleROIBuilder toolbox (http://www.fil.ion.ucl.ac.uk/spm/ext/), we created a mask that included the 3 chosen ROIs. This mask consisted of 3 spheres with a radius of 8 mm corresponding to the 3 ROIs and a total of 758.71 voxels. Within the mask, statistical inferences were made at $P < .05$ after FWE correction for multiple comparisons.

RESULTS

SOCIODEMOGRAPHIC AND CLINICAL PARAMETERS

No statistically significant differences were noted among the UHR-T, UHR-NT, and control groups in age, sex, total GMV, and race/ethnicity (Table 1).

DIFFERENCES BETWEEN THE UHR AND CONTROL GROUPS

The UHR group had less GMV than did controls (at $P < .05$ after FWE correction) in 3 areas of the frontal cortex: the medial orbital gyrus and the gyrus rectus bilaterally and the right anterior cingulate gyrus (**Figure 1** and **Table 2**). In these regions, no significant effect of medication was noted even at trend level ($P < .05$ uncorrected). There were no areas in which UHR individuals had more GMV than did controls.

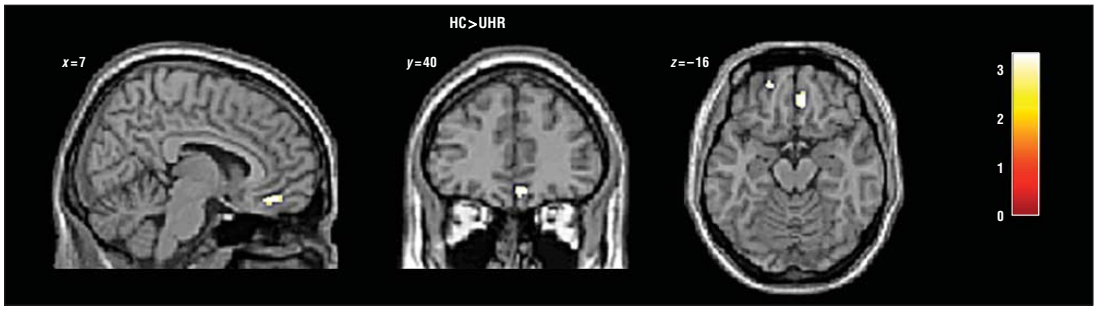

Figure 1. Differences between the ultra-high-risk (UHR) and control (HC) groups. The images show the medial orbital region, where the total UHR sample showed reduced gray matter volume relative to HCs ($P<.05$ after familywise error correction).

Table 2. MNI Coordinates and *z* Scores for Regions Showing Differences in Gray Matter Volume Between the UHR and Healthy Control Group[a]

Healthy Control>UHR Area[b]	Hemisphere	MNI Coordinates			Cluster Size, No. of Voxels	*z* Score	*P* Value
		x	y	z			
Medial orbital gyrus	Right	7	40.5	−16.5	130	5.44	.001
Cingulate gyrus	Right	12	58.5	9	50	5.35	.001
Medial orbital gyrus	Left	−18	52.5	−16.5	30	4.94	.005
Gyrus rectus	Right	3	25.5	−25.5	73	4.94	.01
Gyrus rectus	Left	−4	25.5	−22.5		4.80	.02

Abbreviations: MNI, Montreal Neurological Institute; UHR, ultra-high risk.
[a] $P<.05$ after familywise error correction.
[b] No clusters were detected for the UHR>control contrast.

DIFFERENCES BETWEEN UHR INDIVIDUALS WHO DID AND DID NOT DEVELOP PSYCHOSIS

Analysis by ROI revealed reduced gray matter volume in the UHR-T group relative to the UHR-NT group in the anterior part of the left parahippocampal gyrus (bordering the uncus) (MNI coordinates x=−21, y=6, and z=−27; P=.03; *z*=3.35; and cluster size=6 voxels) (at $P<.05$ after FWE correction) (**Figure 2**), where the difference between UHR-T and UHR-NT accounted for 14% of the total variance. In this region, no significant effect of medication was noted even at trend level ($P<.05$ uncorrected). Plotting of gray matter values revealed that this reduction was evident in each of the 4 sites examined for this contrast (Figure 2). There were no significant differences in the other ROIs (ie, in the right inferior frontal gyrus and the left superior temporal gyrus).

To examine the predictive value of GMV in the left parahippocampal gyrus, we extracted gray matter values from the peak voxel and performed a series of cross-validation analyses using a predictive linear model in SPSS software. This involved developing a predictive model based on a data set from a single scanner site and testing it in each of the other data sets; the average predictive accuracy was 62% (sensitivity=61% and specificity=65%). We also performed a 3-fold cross-validation analysis irrespective of scanner site that involved developing a predictive model based on two-thirds of the total sample and testing it in the remaining one-third; this yielded an average accuracy of 67% (sensitivity=68% and specificity=66%).

COMMENT

We used MRI to study a large sample of UHR individuals created by pooling data from 5 sites. The UHR individuals were observed clinically subsequent to scanning and were subcategorized according to which individuals developed psychosis and which did not.

On the basis of previous MRI studies of smaller UHR samples collected at single sites,[11,15,18,23-27] we first tested the hypothesis that the UHR group as a whole would show volumetric abnormalities relative to controls that were qualitatively similar to those seen in patients with schizophrenia. Consistent with this prediction, the UHR group expressed significant reductions in GMV in the prefrontal and anterior cingulate cortices ($P<.05$ after FWE correction), areas that have been consistently implicated in volumetric neuroimaging studies of schizophrenia.[37] In contrast, we did not identify areas where there was more GMV in the UHR sample than in controls.

The main prediction was that UHR individuals who later developed psychosis would show differences in regional GMV in the inferior frontal, parahippocampal, and superior temporal cortices compared with those who did not become psychotic. This hypothesis was, in part, confirmed: the UHR-T subgroup showed relatively reduced GMV in the anterior part of the left parahippocampal gyrus, bordering the hippocampal uncus ($P<.05$ after FWE correction). In this area, there was less gray matter in UHR-T individuals than in controls ($P<.05$ corrected) but no significant difference between the UHR-NT and

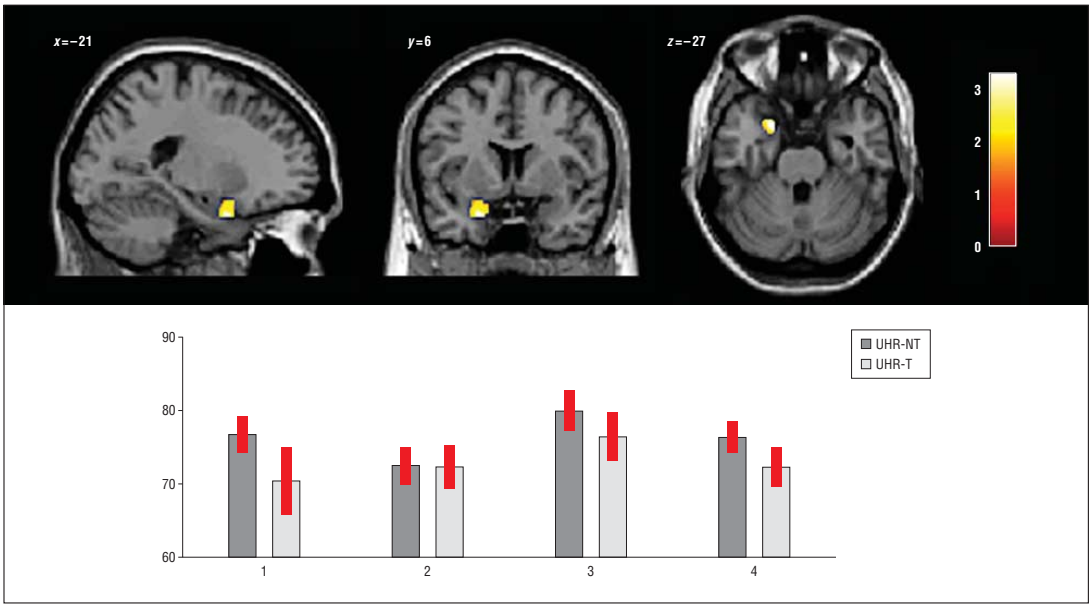

Figure 2. Differences between ultra-high-risk (UHR) individuals who did (UHR-T) and did not (UHR-NT) develop psychosis. The UHR-T individuals had less gray matter volume than did the UHR-NT individuals in the left parahippocampal gyrus, bordering the uncus (MNI [Montreal Neurological Institute] coordinates x, y, and z: −21, 6, and −27, respectively). For visualization purposes, effects are displayed at $P<.05$ uncorrected. The plot shows mean gray matter volumes for the 2 UHR subgroups at each site (x-axis:1 indicates London, United Kingdom; 2, Basel, Switzerland; 3, Munich, Germany; and 4, Melbourne, Australia); values on the y-axis refer to cubic millimeters per voxel. Error bars represent SD.

control groups (eTable 10). A series of cross-validation analyses also revealed that GMV in this region allowed discrimination between UHR-T and UHR-NT with an accuracy of up to 67%. In contrast to some previous single-center studies,[16,36] we did not find statistically significant differences in the inferior frontal and superior temporal gyri.

Reductions in parahippocampal volume have been reported in high-risk individuals relative to controls,[36] as have alterations in parahippocampal function.[38] Over time, reductions in parahippocampal volume have been described in high-risk individuals with transient or isolated psychotic symptoms,[16] and longitudinal reductions have been described in high-risk individuals who developed psychosis.[15] Moreover, cross-sectional comparisons indicate that patients with first-episode psychosis have smaller parahippocampal volumes than do controls and UHR individuals.[26] Meta-analyses suggest that the parahippocampal region is one of the most robust sites of volume reduction in chronic schizophrenia.[37,39-41] Contemporary animal models of psychosis propose that altered parahippocampal activity drives subcortical dopamine dysfunction.[42] These observations are consistent with the notion that the parahippocampal cortex is critically implicated in psychotic disorders.

At the time of scanning, the UHR-T and UHR-NT groups were clinically indistinguishable; the volumetric differences observed between them could be interpreted as neurobiological markers of an especially increased vulnerability to psychosis or as early manifestations of a neuropathologic process underlying the transition to psychosis. Because the data in the present study were collected at a single cross-sectional time point, we cannot determine at what stage these differences first emerged. This issue could be addressed by longitudinal neuroimaging studies of individuals at different time points in the prodromal phase of psychosis.

A limitation of this study is that the data were collected using different scanners and different acquisition sequences.[22,43,44] Nevertheless, we are confident that the results do not represent an artifact due to the use of different scanners for several reasons. First, we sought to control for these effects by using only MRI data collected with T_1-weighted sequences and by modeling scanner site as an independent factor in the statistical analysis. Second, a comparable proportion of controls, nonconverters, and converters was scanned at each site, except for 1 of the London data sets, which was, therefore, excluded from the UHR-T vs UHR-NT comparison. Third, plotting of gray matter values suggested that the differences between the UHR-T and UHR-NT groups were evident not only across different centers but also within each site and, therefore, cannot be explained by interscanner differences (Figure 2). Fourth, when we examined the impact of scanner site, we found no evidence of either scanner effects or scanner × group interactions in regions that differed between groups, even when lowering the statistical threshold to $P<.05$ (uncorrected). Fifth, this approach to the integration of multiscanner data in the same statistical model has been used successfully in previous studies[22,43-45] that also combined different scanners and acquisition sequences; these studies typically found that scanner differences were substantially less than group differences. Consistent with this,

we found that scanner-related variance (11%) was less than group-related variance (14%) in the left parahippocampal region, which differed between the UHR-T and UHR-NT groups. The impact of the use of different scanners and acquisition sequences could be further controlled for by scanning the same individuals at each site, but this was not feasible in the present investigation.

Data from the present study suggest that in the future it may be possible to use MRI to facilitate the prediction of psychosis in those at high risk.[46] This would be particularly useful in the clinical management of individuals at UHR because it is difficult to predict which individuals will go on to develop psychosis on the basis of their clinical features.[4] As a result, it is not possible to focus the provision of clinical resources, such as putatively preventive treatments,[47] to the subgroup of UHR individuals who will later become psychotic. Clinical application of neuroimaging in this context requires the identification of predictive markers at an individual level, whereas the present data represent group differences. Image analysis methods that classify individuals according to patterns of data associated with diagnostic categories may provide a means of addressing this issue.[48]

In conclusion, UHR is associated with alterations in regional GMV, and in this population, reductions in the parahippocampal region may be specifically linked to the later onset of psychosis. These findings suggest that neuroimaging data may facilitate the prediction of illness in individuals at high risk for psychosis and may inform the development of new interventions designed to delay or prevent its onset.

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A1.2. Reduced parahippocampal cortical thickness in subjects at ultra-high risk for psychosis

Candidate's contribution to the study: I have carried out the preprocessing and the statistical analyses of the MRI data. I have drafted the first version of the manuscript, I have implemented the suggestions made by the co-authors and I have revised the manuscript according to the reviewers' comments during the publication phase.

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ORIGINAL ARTICLE

Reduced parahippocampal cortical thickness in subjects at ultra-high risk for psychosis

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Background. Grey matter volume and cortical thickness represent two complementary aspects of brain structure. Several studies have described reductions in grey matter volume in people at ultra-high risk (UHR) of psychosis; however, little is known about cortical thickness in this group. The aim of the present study was to investigate cortical thickness alterations in UHR subjects and compare individuals who subsequently did and did not develop psychosis.

Method. We examined magnetic resonance imaging data collected at four different scanning sites. The UHR subjects were followed up for at least 2 years. Subsequent to scanning, 50 UHR subjects developed psychosis and 117 did not. Cortical thickness was examined in regions previously identified as sites of neuroanatomical alterations in UHR subjects, using voxel-based cortical thickness.

Results. At baseline UHR subjects, compared with controls, showed reduced cortical thickness in the right parahippocampal gyrus ($p<0.05$, familywise error corrected). There were no significant differences in cortical thickness between the UHR subjects who later developed psychosis and those who did not.

Conclusions. These data suggest that UHR symptomatology is characterized by alterations in the thickness of the medial temporal cortex. We did not find evidence that the later progression to psychosis was linked to additional alterations in cortical thickness, although we cannot exclude the possibility that the study lacked sufficient power to detect such differences.

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Key words: Cerebral cortex, magnetic resonance imaging, parahippocampal gyrus, prodromal period, schizophrenic psychoses, voxel-based cortical thickness.

Introduction

The first episode of a psychotic disorder is usually preceded by a prodromal period characterized by a progressive decline in functioning and the emergence of attenuated psychotic symptoms. Individuals with these clinical features are said to be at 'ultra-high risk' (UHR) for psychosis because 18–36% of them will develop a psychotic disorder within 3 years (Fusar-Poli *et al.* 2012). Early clinical intervention in the UHR population may reduce the risk of later transition to psychosis and improve long-term clinical and functional outcome (McGorry *et al.* 2009); however, at present it is difficult to predict based on first clinical presentation which UHR individual who will and will not go on to develop psychosis. Recent work has sought to examine if neuroimaging can be used to identify individuals at UHR and predict who will subsequently make transition to psychosis within this population (Koutsouleris *et al.* 2009).

Studies using voxel-based morphometry (VBM) have examined whether UHR subjects are affected by neuroanatomical abnormalities. Cross-sectional studies indicate that, relative to healthy controls, UHR subjects

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have reduced grey matter (GM) volume in frontal (Meisenzahl *et al.* 2008; Mechelli *et al.* 2011), lateral and medial temporal regions (Meisenzahl *et al.* 2008). Studies that used a region of interest (ROI) approach reported GM volume increases (Buehlmann *et al.* 2010) but also reductions (Phillips *et al.* 2002) in the hippocampus, reductions in the planum polare/temporale, insula and superior temporal gyrus (Takahashi *et al.* 2009, 2010), increases in the pituitary volume (Büschlen *et al.* 2011), and reductions in the anterior cingulate cortex (ACC) (Röthlisberger *et al.* 2012) in the UHR group compared with healthy controls.

In VBM studies, relative to UHR subjects who did not develop psychosis (UHR-NT), those who later became psychotic (UHR-T) had reduced volumes in the inferior frontal cortex, medial and lateral temporal cortex, ACC (Pantelis *et al.* 2003), insular, inferior and superior frontal cortex (Borgwardt *et al.* 2008). ROI studies suggest that later transition to psychosis is associated with reduced volume in the left parahippocampal gyrus (Mechelli *et al.* 2011), the insula bilaterally (Takahashi *et al.* 2009), the left ACC (Röthlisberger *et al.* 2012), and with increased pituitary (Büschlen *et al.* 2011) and hippocampus (Buehlmann *et al.* 2010) volumes.

Some ROI studies did not find any significant differences between UHR subjects and healthy controls (Wood *et al.* 2005; Velakoulis *et al.* 2006) or between UHR-T and UHR-NT individuals (Yucel *et al.* 2003; Velakoulis *et al.* 2006). Nevertheless, evidence that there may be volumetric differences between the latter subgroups raises the possibility that neuroimaging measures might be able to facilitate the prediction of clinical outcome in UHR subjects.

The vast majority of structural neuroimaging studies in UHR individuals have focused on GM volume. However, neuroanatomical alterations in psychosis may be expressed not only in terms of GM volume but also as subtle changes in cortical thickness. While the analysis of GM volume returns a mixed measure that depends on local cortical thickness as well as cortical folding and gyrification (i.e. cortical surface area), the analysis of cortical thickness is considered to specifically target the presence of cortical atrophy (Hutton *et al.* 2009). Therefore the two approaches provide complementary information and one can be more sensitive than the other depending on the process underlying neuroanatomical changes. Cortical thickness in the human brain can be examined using the automated data analysis of T_1-weighted images (Hutton *et al.* 2008). Application of this approach in patients with first-episode and established schizophrenia suggests that there is a cortical thinning in the anterior cingulate (Narr *et al.* 2005*a*; Fornito *et al.* 2008*a*; Schultz *et al.* 2010*b*), prefrontal (Narr *et al.* 2005*a*; Venkatasubramanian *et al.* 2008; Schultz *et al.* 2010*b*), temporal (Narr *et al.* 2005*a*; Fornito *et al.* 2008*a*) and occipital (Narr *et al.* 2005*b*) cortices. In UHR subjects, reduced cortical thickness has been reported in the prefrontal, ACC, inferior parietal, superior temporal and parahippocampal cortices compared with healthy controls (Jung *et al.* 2011). One study has reported that in UHR individuals who subsequently developed psychosis the ACC was thinner than in UHR-NT (Fornito *et al.* 2008*b*) and one has reported a progressive thinning in the anterior cingulate, precuneus and temporo-parietal-occipital cortex compared with UHR-NT and healthy controls (Ziermans *et al.* 2012). Another study did not find differences in cortical thickness between individuals at UHR, patients with a first psychotic episode and healthy controls, but the groups differed in cortical thickness asymmetry (Haller *et al.* 2009).

The findings from studies of cortical thickness in UHR subjects have thus been inconclusive, which may reflect the relatively small sample sizes examined to date. UHR subjects are difficult to recruit and it is therefore a significant challenge for any single centre to scan a large sample. Multi-centre studies provide a means of addressing this issue with the pooling of data from different sites to produce relatively large samples.

We adopted this approach in the present study. Our aim was to combine magnetic resonance imaging (MRI) data from multiple centres and measure cortical thickness in large samples of UHR subjects and healthy controls. We also sought to compare cortical thickness in UHR subjects who subsequently did or did not developed psychosis. Whole-brain MRIs were acquired from individuals at UHR for psychosis and healthy controls at four psychiatric research centres in London, Basel, Munich and Melbourne. At each site, subjects were scanned at first clinical presentation, and followed up clinically or in the context of research projects for at least 2 years, so that they could be subcategorized according to psychosis outcome. The MRI data from each site were combined to form a large UHR sample, which was subdivided into subjects who later had developed psychosis and subjects who had not. The thickness of the cerebral cortex was assessed using a voxel-based cortical thickness (VBCT) approach that generates maps from MRI data in which each voxel in the GM is assigned a thickness value and regionally specific differences are compared on a voxel-by-voxel basis (Hutton *et al.* 2008, 2009). This method differs from others reported in the literature, as it does not require the construction of a three-dimensional model for extracting cortical thickness values. For instance, surface-based techniques involve

the generation of surface models that are driven by image information and surface geometry to fit the GM and white matter (WM) surfaces of the image (Fischl & Dale, 2000; Hutton *et al.* 2008). Cortical thickness is subsequently defined at surface points and is computed based on the measure of the distance between them. Another approach involves extracting only the surface between the GM and the WM and then mapping towards the surface the thickness values that are derived by calculating the distance between voxels in the cortex and the surface (Lerch & Evans, 2005; Hutton *et al.* 2008). In contrast, using the VBCT technique, GM and WM boundaries are defined on the basis of whole voxel information (Jones *et al.* 2000; Hutton *et al.* 2008) and cortical thickness is calculated at every volumetric point within the cortex and based on the length of the trajectory from one boundary to another (Hutton *et al.* 2008).

Our first prediction, based on data from previous studies (Narr *et al.* 2005*a*; Fornito *et al.* 2008*a*,*b*; Schultz *et al.* 2010*b*; Jung *et al.* 2011; Ziermans *et al.* 2012), was that the UHR group as a whole would show differences in cortical thickness relative to controls in areas that have previously been identified in studies of cortical thickness in UHR subjects and patients with first-episode psychosis: the frontal, anterior cingulate, parahippocampal, temporal, parietal cortices and the precuneus. Our final prediction was that within the UHR sample, subjects who developed psychosis subsequent to scanning would show more pronounced cortical thickness abnormalities in these regions (Narr *et al.* 2005*a*; Fornito *et al.* 2008*a*,*b*; Schultz *et al.* 2010*b*; Jung *et al.* 2011; Ziermans *et al.* 2012) than those who did not.

Method

Sample

All the UHR subjects were recruited from specialized clinical services for this group in London [Outreach and Support in South London (OASIS)], Basel [Clinic for Early Detection of Psychosis (FEPSY) at the Psychiatric Outpatient Department, University Hospital], Melbourne [Personal Assessment and Crisis Evaluation (PACE) Clinic] and Munich [Early Detection and Intervention Centre for Mental Crisis (FETZ), Department of Psychiatry and Psychotherapy, Ludwig-Maximilians-University]. Criteria used to identify participants at UHR for psychosis were comparable across the different sites as reported in Supplementary Table S2. Specifically, the London and Melbourne sites used as a screening instrument the Comprehensive Assessment for at Risk Mental State (Yung *et al.* 2005); the Basel site used as a screening assessment the Basel Screening Instrument for Psychosis (Riecher-Rössler *et al.* 2008); and the Munich site used the Bonn Scale for the Assessment of Basic Symptoms (Gross *et al.* 1987). In addition they also assessed attenuated psychotic symptoms and brief limited intermittent psychotic symptoms as defined by the PACE criteria (Yung *et al.* 2005). Data were combined from these four sites. This sample overlaps with a multi-centre sample that was previously used to investigate GM volume in UHR subjects (Mechelli *et al.* 2011), and includes subjects that participated in previous single-centre studies of GM volume in this population. In total there were MRI data from 167 UHR subjects. MRI data acquired from healthy volunteers from the same geographical area as the UHR subjects at each site were combined to form a control dataset. Healthy controls were excluded if there was a past or present personal or familiar history of neurological and/or psychiatric conditions. The total control sample comprised 150 subjects, and was comparable with the total UHR sample with respect to gender, age and ethnicity (Table 1).

Subsequent to MRI scanning, the UHR subjects were followed up and assessed regularly for at least 2 years at all four sites. UHR subjects who developed a first episode of psychosis during this period were identified using standardized transition criteria (McGorry *et al.* 2003; Yung *et al.* 2004). Each site used this information to subdivide their UHR sample into a group that had made a transition to psychosis (UHR-T), and a group that had not (UHR-NT). In the following 30.6 (S.D. = 10.4) months, 50 (30%) of the UHR individuals developed psychosis (UHR-T) and 117 did not (UHR-NT). Transition to psychosis during the follow-up period was established according to Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria based on clinical consensus between at least two experienced psychiatrists. Most of the UHR group (147/167, 84%) had never taken antipsychotics at time of scanning; 20 (12%) had been exposed to antipsychotics; the mean antipsychotic medication exposure time was 7.5 (S.D. = 11.1) weeks. UHR participants that were receiving medication were scanned at the London or Basel sites. Inclusion and exclusion criteria for the two experimental groups, clinical characteristics and sociodemographic of the UHR subjects are reported in the Supplementary material (Supplementary Tables S1–3, S5–8).

Image acquisition

At all four sites, volumetric MR images were acquired using scanner field strengths of 1.5 T and a T_1-weighted protocol. Two sites used General Electric

Table 1. *Sociodemographic data of the study samples*

Characteristics	Healthy controls (n=150)	UHR-NT (n=117)	UHR-T (n=50)	Significance
Mean age, years (S.D.)	23.4 (4.3)	23.3 (5.3)	22.9 (4.6)	$F_{2,314}$=0.388, df=2, p=0.6979
Gender, n				χ^2=4.188, df=2, p=0.123
Female	51	49	13	
Male	99	68	37	
Ethnicity, n				
Caucasian	128	96	43	χ^2=8.320, df=6, p=0.216
Black	7	5	1	
Asian	9	5	0	
Mixed	6	11	6	
Handedness, n				
Right	132	111	45	χ^2=4.164, df=4, p=0.384
Left	12	4	4	
Ambidextrous	6	2	1	
Medication, n	N/A	21	5	χ^2=1.684, df=1, p=0.194

UHR-NT, Ultra-high risk without disease transition; UHR-T, UHR with disease transition; S.D., standard deviation; df, degrees of freedom; N/A, not applicable.

scanners (i.e. London and Melbourne) and two used Siemens scanners (i.e. Basel and Munich). The details of the image acquisition sequence are reported in Supplementary Table S4.

Data analysis

Sociodemographics

Sociodemographic differences between groups were examined using one-way analysis of variance for parametric data, and by χ^2 test for non-parametric data, as implemented in SPSS 19.0 for Windows (IBM, USA) (Table 1, Supplementary Tables S5–8).

Pre-processing

Pre-processing for the analysis of cortical thickness was carried out using the procedure described by Hutton *et al.* (2008, 2009). In brief, all the images were visually checked and re-sampled to a voxel size of 1 mm^3 using trilinear interpolation. Using the unified segmentation procedure implemented in SPM8 (http://www.fil.ion.ucl.ac.uk/spm), the images were segmented into GM, WM and cerebrospinal fluid (CSF) (Ashburner, 2007). For each subject, this resulted in a set of three images in the same space as the original T_1-weighted image, in which each voxel was assigned a probability of being GM, WM and CSF, respectively. A VBCT map was created for each subject using the GM, WM and CSF segments created in the previous step (Hutton *et al.* 2008). This method is implemented as a toolbox for SPM (Hutton *et al.* 2008, 2009). In brief, it uses the input tissue probability maps and a transformed labelled brain atlas (http://www.fil.ion.ucl.ac.uk/spm/ext/#IBASPM). Starting from the initial estimate of the GM/WM boundary, layers of one voxel in thickness are successively added to surround the WM allowing voxels to be identified where the GM from different sides of a sulcus was in contact. Once all GM voxels have been processed in this way, Laplace's equation is solved for all voxels between the final GM/WM and GM/CSF boundaries resulting in a scalar field that makes a smooth transition from one boundary to the other. The gradient of this field at each point forms a unique trajectory connecting the two boundaries, and the thickness at each point is calculated by integrating along these trajectories. The resulting VBCT maps are created in the space of the original input images which contain cortical thickness values within voxels identified as cortical GM and zeros outside the cortex. DARTEL (Ashburner, 2007), an algorithm for diffeomorphic image registration which is implemented as a toolbox for SPM8, was used to warp the VBCT maps into a new group-specific reference space representing an average of all the subjects. This procedure uses the GM and WM segments estimated from the original T_1-weighted images to calculate a group-specific template and the deformation fields required to warp data from each subject to the new template. Each VBCT map was warped to the new template using the corresponding subject-specific deformation field and was re-sampled to an isotropic voxel size of 1.5 mm^3 using trilinear interpolation. The warped VBCT maps were scaled by the Jacobian

determinant of the deformations to account for stretching and compression and subsequently smoothed with a 6 mm Gaussian kernel then divided by a binary mask of each original VBCT map which had been identically warped, scaled and smoothed (see Supplementary Fig. S1). A Gaussian kernel of 6 mm was chosen because, when investigating cortical thickness, it is important to keep smoothing to a minimum so that any abrupt changes in thickness that may occur at the boundaries between cortical areas are not obscured (Hutton *et al.* 2008). This procedure results in smoothed warped VBCT maps for which the Gaussian smoothing kernel applied in the warped space has been projected into the native space of the subject, and the cortical thickness values are preserved over a region the size of the smoothing kernel.

Statistical analysis

Statistical analysis was performed using SPM8 software (Wellcome Trust Centre for Neuroimaging, UK) on the smoothed warped VBCT maps. An analysis of variance was used to compare cortical thickness in UHR-T, UHR-NT and healthy control subjects. Scanner site was modelled as an additional factor, resulting in a total of 12 experimental groups. Including scanner site as a factor in the statistical analysis allowed us to model scanner-related variance in the data, which had the effect of reducing error variance and increasing statistical sensitivity. We also modelled age, gender, ethnicity and handedness as covariates of no interest to minimize any confounding effect of these variables on the findings. An ROI approach was used to examine between-group differences in areas where abnormalities in cortical thickness have been identified in MRI studies of individuals at UHR or with a first episode of psychosis, excluding studies that included data from subjects who participated in the present investigation. These comprised the parahippocampal gyrus, inferior frontal gyrus, anterior cingulate, superior temporal gyrus, inferior parietal gyrus and the precuneus (Narr *et al.* 2005*a*; Schultz *et al.* 2010*b*; Jung *et al.* 2011; Ziermans *et al.* 2012). Using WFU PickAtlas (http://www.nitrc.org/projects/wfu_pickatlas/) we created a mask that included the six chosen ROIs and comprised a total of 11 700 voxels. Within this mask, statistical inferences were made using a statistical threshold of $p<0.05$ after familywise error (FWE) correction for multiple comparisons as calculated in SPM8. Trends that did not survive correction for multiple comparisons ($p<0.001$ uncorrected) are reported but not discussed. For completeness we also performed a whole-brain analysis using a statistical threshold of $p<0.05$ after FWE correction for multiple comparisons.

Results

Sociodemographics

No statistically significant differences were noted among the UHR-T, UHR-NT and control groups in age, gender, ethnicity and handedness (Table 1).

Differences in cortical thickness between UHR subjects and healthy controls

Within the ROIs, the cortex was thinner in the UHR group than in controls in the right parahippocampal gyrus [$p<0.05$ FWE corrected; z score=4.06; Montreal Neurological Institute (MNI) coordinates x=25, y=–4, z=–13; see Fig. 1]. There was also a trend ($p<0.001$ uncorrected) for a thinner cortex in the UHR group compared with controls in the inferior part of the left parahippocampal gyrus (z score=3.42; MNI coordinates x=–20, y=–7, z=–30). Plotting of the cortical thickness values revealed that the reduction in right parahippocampal cortical thickness was evident in the data from each of the four sites (Fig. 1). In contrast, there were no areas in which UHR individuals had a thicker cortex than healthy controls. The whole-brain analysis did not identify significant differences in cortical thickness between the UHR group and healthy controls at $p<0.05$ (FWE corrected).

Differences in cortical thickness between the UHR-T and UHR-NT groups

No differences were observed for the comparison between the UHR-T and UHR-NT groups at $p<0.05$ after FWE correction. At a less conservative statistical threshold ($p<0.001$ uncorrected), there was a trend for cortical thinning in the UHR-T group in the orbital part of the left inferior frontal gyrus (z score=3.32; MNI coordinates x=–33, y=32, z=–15). The whole-brain analysis revealed no significant differences between the UHR-T and UHR-NT groups at $p<0.05$ (FWE corrected).

Discussion

Previous neuroimaging studies have reported cortical thinning in schizophrenia, as well as in first-episode psychosis and in subjects at UHR for psychosis (Narr *et al.* 2005*a*; Fornito *et al.* 2008*a*,*b*; Schultz *et al.* 2010*b*; Jung *et al.* 2011; Ziermans *et al.* 2012). However, the results of these studies have not always been consistent; this may reflect the recruitment of relatively small samples, the use of different study designs, and the investigation of samples that were heterogeneous with respect to age, duration of illness and exposure to treatment. In the present study, we sought to reduce the impact of these potential methodological

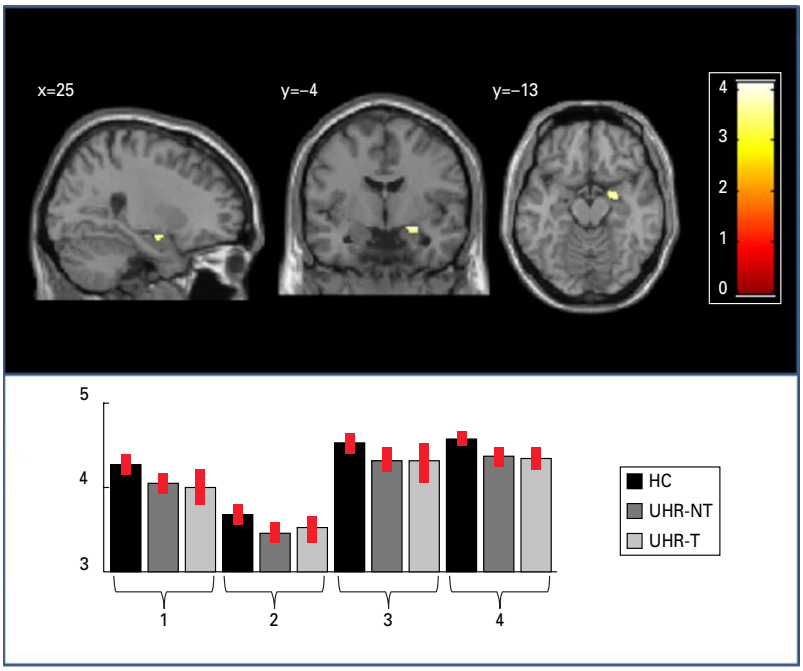

Fig. 1. Cortical thickness differences between the ultra-high risk (UHR) and healthy control (HC) groups: right parahippocampal region where the total UHR sample showed cortical thinning relative to HCs ($p<0.05$ after familywise error correction). For visualization purposes, effects are displayed at $p<0.001$ uncorrected. The plot shows cortical thickness values for the HC group and the two UHR subgroups (UHR-T, transition to psychosis; UHR-NT, no transition to psychosis) at each site (x axis: 1=London; 2=Basel; 3=Melbourne; 4=Munich); values on the y axis refer to millimetres. Error bars represent standard deviations.

pitfalls by assessing cortical thickness in a relatively large sample of individuals at UHR for psychosis, all of whom were scanned at a similar stage of illness, when they first presented with clinical high-risk symptoms. Most of the sample had not been treated before. Our first hypothesis was that the UHR group as a whole would show differences in regional cortical thickness relative to controls, and that these would be most evident in areas that have previously been identified as sites of cortical thickness or volume abnormalities in studies of UHR and first-episode subjects (i.e. the temporal, frontal, parietal, anterior cingulate, parahippocampal cortices and the precuneus). This hypothesis was in part confirmed, in that we found that the right parahippocampal cortex was thinner in the UHR group than in controls. This finding is consistent with those of a recent investigation which also found reductions in thickness in other cortical areas (Jung *et al.* 2011). Our result is also in line with evidence that the density of the parahippocampal gyrus is altered in UHR and familial high-risk subjects (Job *et al.* 2003). Furthermore, in first-episode schizophrenia, altered right parahippocampal–lingual cortical folding and reduced cortical thickness have been found (Schultz *et al.* 2010*a*). Finally, this region has also been identified as a site of functional (Allen *et al.* 2011) alterations in UHR subjects and is one of the most robust site of volume reduction (Seidman *et al.* 2003) and neuropathological abnormalities (Shenton *et al.* 2001) in schizophrenia.

In our previous volumetric study in an UHR sample that overlapped with that in the present study, we found differences between the UHR sample and controls in the ventral prefrontal cortex and ACC, but not in the parahippocampal cortex (Mechelli *et al.* 2011). However, in that study the subgroup of UHR subjects that subsequently developed psychosis had smaller left parahippocampal volumes than the UHR subjects who did not make a transition to psychosis. This volumetric finding was in a similar part of the parahippocampal gyrus to the site of the difference in cortical thickness between all UHR subjects and controls in the present study, although in the opposite hemisphere. Differences in the location of alterations in cortical thickness and cortical volume may reflect the fact that abnormalities in these two measures are

not necessarily manifestations of the same underlying neuroanatomical changes. This would be consistent with recent evidence that cortical thickness and surface area are genetically and phenotypically independent and that the local cortical volume is more closely related to surface area than to cortical thickness (Winkler *et al.* 2010). Therefore differences between the results of our present study and our previous VBM study may be partially explained by the fact that the cortical thickness method does not take surface information into account. The volumetric reduction of the left parahippocampal gyrus reported in our VBM study (Mechelli *et al.* 2011) may denote a marker of progression consistent with the results of earlier investigations (Pantelis *et al.* 2003; Job *et al.* 2005), whereas the thinning of the right parahippocampal gyrus may represent either a vulnerability marker (evident not only in the UHR stage but also in the earlier asymptomatic stage) or a specific marker of the UHR stage (not evident in the earlier asymptomatic stage). These two alternative options could be investigated by acquiring longitudinal neuroimaging data from individuals at high genetic risk.

Our second hypothesis was that UHR subjects who went on to develop psychosis would already at baseline show more pronounced cortical thickness abnormalities than UHR subjects who did not. We did not find any differences that survived correction for multiple comparisons. Two previous studies have investigated cortical thickness in UHR subjects in relation to clinical outcome. One study, which restricted its analysis to the ACC, reported cortical thinning in this region in the UHR-T group compared with the UHR-NT group (Fornito *et al.* 2008*b*). We included the ACC as one of our ROIs but did not replicate this finding, although this may be in part explained by methodological differences in thickness measurements and in ROIs investigated. The second study used MRI to scan UHR subjects at two time points and compared longitudinal changes in cortical thickness in UHR individuals who did and did not make a transition to psychosis. The UHR-T group showed longitudinal reductions in several regions including anterior cingulate, precuneus and temporo-parietal-occipital areas compared with controls, whereas the UHR-NT group did not (Ziermans *et al.* 2012). In contrast, no differences in cortical thickness were reported between UHR-T and UHR-NT subjects at baseline. The inconsistency between previous studies and our investigation could be in part explained by the use of different techniques to measure cortical thickness. In particular, the studies by Fornito and colleagues and Ziermans and colleagues used two different surface-based methods (Fischl & Dale, 2000; Kim *et al.* 2005; Fornito *et al.* 2008*b*; Ziermans *et al.* 2012) while in the present investigation, a voxel-based method was used to derived cortical thickness values (Hutton *et al.* 2008). Another study reported no baseline differences in cortical thickness between UHR and first-episode psychosis and healthy controls, although the analysis of cortical asymmetry revealed significant group differences (Haller *et al.* 2009). The lack of significant differences between UHR-T and UHR-NT subjects may seem surprising, given that the present sample was relatively large, and abnormalities in functional and in other structural measures between these subgroups have previously been identified (Pantelis *et al.* 2003; Borgwardt *et al.* 2008, Mechelli *et al.* 2011; Allen *et al.* 2012). One possible explanation for the absence of significant differences in the present study is that there was some heterogeneity within our UHR sample. Individuals can meet the UHR inclusion criteria through different patterns of clinical and cognitive symptoms, and it is unknown if these reflect distinct pathophysiological processes. Unfortunately, a stratification of the statistical analysis by type of UHR inclusion criteria was not possible in the present investigation, as incorporating this additional factor would require much larger single-centre sample sizes. The sample may also have been clinically heterogeneous with respect to the co-morbid anxiety, depression and substance abuse that are often present in UHR subjects (Svirskis *et al.* 2005).

Another possibility is that some subjects who met transition criteria subsequently have returned to lower levels of psychotic symptoms and higher levels of functioning, while some who did not meet criteria for transition actually have deteriorated in functioning over time. This latter group may be more likely to develop schizophrenia than the former group (Yung *et al.* 2010). Thus, the non-transitioned but low functioning group may show brain imaging changes consistent with the ones observed in schizophrenia, but the high functioning transitioned cases may not.

The age range of our UHR individuals varied considerably across the four sites (age range 15–37 years). Previous work indicates that the pattern of neuroanatomical findings in schizophrenia with a relatively early onset (e.g. before the age of 18 years) differs from that in schizophrenia with onset in adulthood and that the longitudinal trajectory of brain abnormalities varies with the age of onset (Gogtay *et al.* 2011). Although we modelled age as a covariate of no interest in the statistical analysis, the fact that the UHR sample comprised individuals at different stages of brain development might have reduced the likelihood of detecting reliable differences.

A further potential contributory factor is that the MRI data we studied were collected on different scanners, using different acquisition sequences. However,

we are confident that our results were not significantly affected by the use of different scanners for several reasons. First, only MRI data collected with T_1-weighted sequences were used and scanner site was modelled as an independent factor in the statistical analysis. Second, a comparable proportion of control, UHR-NT and UHR-T subjects were scanned at each scanning site. Third, plotting of GM values suggested that the differences between UHR and healthy control groups were evident also within each site and therefore cannot be explained by inter-scanner differences (Fig. 1). Finally, the present approach to the integration of multi-scanner data has been employed successfully in previous studies that also combined different scanners and acquisition sequences (Stonnington *et al.* 2008; Segall *et al.* 2009; Suckling *et al.* 2010). Nevertheless, we cannot completely exclude an effect of scanner on the findings, and ideally multi-centre studies should employ the same acquisition sequence, and calibrate data across scanners using phantoms and common subjects to minimize the risk of scanner-related effects (Jack *et al.* 2008).

The whole-brain analysis did not identify significant differences in cortical thickness between the UHR group and healthy controls or between UHR-T and UHR-NT subjects at $p<0.05$ (FWE corrected). This could be in part explained by the heterogeneity of the UHR sample (e.g. symptoms, level of functioning, age), or by the use of different scanners and sequences. Nevertheless, when restricting the analysis to ROIs, the method employed was able to detect a thickness reduction in the right parahippocampal gyrus in the UHR group compared with the control group at a statistical threshold of $p<0.05$ corrected (FWE). The reduction was evident across all the different scanning sites. Using the whole-brain approach did not enable us to detect differences at a corrected level; this might indicate that group differences in the UHR population are distributed and diluted across the brain cortex.

In conclusion, we observed right parahippocampal thinning in subjects at UHR for psychosis, suggesting that thickness reduction in this region is related to the UHR symptomatology rather than the onset of psychosis. This cortical alteration could represent a marker of vulnerability to psychosis or, alternatively, a specific and distinctive marker of the UHR stage. No reliable differences in cortical thickness were found between subjects who did and did not go on to develop psychosis. Future multi-centre work in this area would benefit from the subcharacterization of UHR individuals with different clinical and cognitive profiles, the recruitment of participants at the same stage of neurodevelopment and the use of a standardized image acquisition sequence.

Supplementary material

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0033291713000998.

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Declaration of Interest

None.

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A1.3. Using structural neuroimaging to make quantitative predictions of symptom progression in individual at ultra-high risk for psychosis

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Using structural neuroimaging to make quantitative predictions of symptom progression in individuals at ultra-high risk for psychosis

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Neuroimaging holds the promise that it may one day aid the clinical assessment of individual psychiatric patients. However, the vast majority of studies published so far have been based on average differences between groups, which do not permit accurate inferences at the level of the individual. We examined the potential of structural Magnetic Resonance Imaging (MRI) data for making accurate quantitative predictions about symptom progression in individuals at ultra-high risk for developing psychosis. Forty people at ultra-high risk for psychosis were scanned using structural MRI at first clinical presentation and assessed over a period of 2 years using the Positive and Negative Syndrome Scale. Using a multivariate machine learning method known as relevance vector regression (RVR), we examined the relationship between brain structure at first clinical presentation, characterized in terms of gray matter (GM) volume and cortical thickness (CT), and symptom progression at 2-year follow-up. The application of RVR to whole-brain CT MRI data allowed quantitative prediction of clinical scores with statistically significant accuracy (correlation = 0.34, $p = 0.026$; Mean Squared-Error = 249.63, $p = 0.024$). This prediction was informed by regions traditionally associated with schizophrenia, namely the right lateral and medial temporal cortex and the left insular cortex. In contrast, the application of RVR to GM volume did not allow prediction of symptom progression with statistically significant accuracy. These results provide proof-of-concept that it could be possible to use structural MRI to inform quantitative prediction of symptom progression in individuals at ultra-high risk of developing psychosis. This would enable clinicians to target those individuals at greatest need of preventative interventions thereby resulting in a more efficient use of health care resources.

Keywords: relevance vector regression, magnetic resonance imaging, cortical thickness, ultra-high risk, psychosis, symptom progression, prediction

INTRODUCTION

The first full-blown psychotic episode is usually preceded by a prodromal phase which is characterized by a progressive decline in functioning and the emergence of attenuated psychotic symptoms. Individuals with these clinical features are said to be at ultra-high risk (UHR) for developing psychosis. Results from a recent meta-analysis suggest that about 18–36% of the UHR population will develop a psychotic disorder within 3 years from first clinical presentation (1). Thus, the study of the UHR population offers a window into the early stages of the illness under minimal influence of confounding factors such as medication and chronicity, and may inform the development of new early interventions aimed at delaying or preventing the onset of the illness.

Neuroimaging offers a promising translational tool for the characterization of brain abnormalities in individuals at UHR for psychosis; in particular, it has been suggested that neuroanatomical and neurofunctional measures could eventually be used to make individualized predictions of clinical outcome in this population. Consistent with this notion, a growing number of studies using structural Magnetic Resonance Imaging (MRI) have identified neuroanatomical differences between individuals at UHR who subsequently did and did not develop psychotic symptoms. Below we provide a brief overview of these studies, and then report the results of a novel investigation that examined whether structural MRI allows accurate quantitative predictions about symptom progression in individuals at UHR for psychosis.

Structural MRI has revealed a number of neuroanatomical differences at first clinical presentation between individuals who subsequently make transition to psychosis (UHR-T) and those who do not (UHR-NT). In whole-brain voxel-based morphometry (VBM) studies, UHR-T relative to UHR-NT subjects showed reduced gray matter (GM) volume of the inferior frontal cortex, medial and lateral temporal, anterior cingulate cortex (ACC), insular, inferior and superior frontal cortices (2), and reduced GM density of the left temporal lobe and right cerebellum (3). In addition, VBM studies employing a region of interest (ROI) approach

indicated that individuals who subsequently make transition to psychosis show reduced volume in the left parahippocampal gyrus (4), the bilateral insula (5) and the left ACC (6), and increased volume of the pituitary gland (7, 8) and the hippocampus (9). A recent VBM investigation has also shown that, in individuals at UHR for psychosis, lower scores on a semantic fluency task are associated with reduced GM density in a distributed network including the right superior/middle temporal gyrus, the right insula, and the left ACC, suggesting that the combination of these two types of data could inform outcome prediction in this population (10).

Neuroanatomical alterations in psychosis may be expressed not only in terms of alterations in GM volume or density but also as changes in regional cortical thickness (CT) (11–15) and the degree of CT asymmetry (16). Consistent with this notion, UHR-T compared to UHR-NT have been found to show cortical thinning of the ACC (17). However, a few subsequent ROI studies did not find any significant differences in CT between individuals who subsequently did and did not make conversion to psychosis (9, 18–23).

While the above studies used a cross-sectional design, a number of investigations have employed a within-subject design to examine the neuroanatomical changes that occur in individuals at UHR around the time of illness onset. These studies have reported progressive reductions in the GM volume of the orbitofrontal and cerebellar cortices (2, 24), fusiform and parahippocampal cortices and cingulate gyrus (24); superior frontal, inferior temporal, superior parietal cortices, and precuneus (2) in UHR-T compared to UHR-NT. In addition, using an ROI approach, within-subject studies have found greater progressive reductions in the GM volume of the insula (5), planum polare, planum temporale, and caudal region (22) in UHR-T compared to UHR-NT (22). Using cortical pattern matching techniques, Sun and colleagues (25) have also revealed volumetric reductions in the prefrontal cortex in UHR-T compared to UHR-NT (25). With respect to CT, the only longitudinal study published so far has reported progressive thinning of the anterior cingulate, precuneus, and temporal-parieto-occipital cortex in UHR-T compared to UHR-NT and healthy controls (26).

A small fraction of studies of the UHR population have investigated white matter (WM) abnormalities associated with transition to psychosis using either VBM or diffusion tensor magnetic resonance imaging (DTI). With respect to VBM, only two studies have investigated WM abnormalities in UHR subjects as a function of clinical outcome (26, 27). In the first study, UHR-T subjects showed increased WM volume in the left frontal lobe and a progressive decrease in the left fronto-occipital fasciculus (27). In the second study, UHR-T subjects showed a decrease in total WM volume relative to healthy controls but not relative to UHR-NT, in addition to which the comparison between UHR-NT and controls was also not significant (26). With respect to DTI, a large number of studies have compared individuals at UHR against healthy controls (28–34) but only three of them have subdivided the UHR group according to clinical outcome (30–32). One of these studies revealed that UHR-T had lower fractional anisotropy (FA), an index of WM integrity, at baseline compared to healthy controls in the medial frontal region (30). In addition, UHR-T had lower FA in the WM lateral to the right putamen and in the left superior temporal gyrus but higher FA in the left posterior temporal WM, compared to UHR-NT (30). Finally, in UHR-T, the FA in the left middle temporal lobe was negatively associated with the severity of positive symptoms (30). The remaining two studies reported no cross-sectional differences in WM integrity between UHR-T and UHR-NT (31, 32). However, Carletti and colleagues (31) reported a progressive reduction of left frontal WM in UHR-T which was not evident in UHR-NT (31).

Taken collectively, the above studies provide evidence for differences in brain structure between individuals at ultra-high risk for psychosis who subsequently do and do not develop the illness, particularly in the prefrontal and temporal cortices. These studies, however, each reported significant effects only at a group level, whereas clinicians treating psychosis have to make decisions about the individual in front of them. Because effects that are significant at a group level do not necessarily permit accurate inferences about individuals, the translational potential of the above findings for everyday clinical practice is unclear. In addition, these studies were conducted using a standard univariate analytical approach in which each voxel is considered independently. This approach is well suited to detect effects that are robust and localized; however, it is not very sensitive to differences that are subtle and highly distributed across the brain. For these reasons, an increasing number of recent studies of psychiatric disorders have adopted an alternative approach based on multivariate machine learning methods (35, 36). A key benefit of multivariate machine learning methods is that they allow one to make predictions that are specific to a given individual, rather than providing an average estimate for a group. This greatly increases the likelihood that the results can be translated into a tool that is useful in a real world clinical setting. A further benefit of multivariate machine learning methods is that they take into account the inter-relationship between different measures (e.g., GM volume across different voxels), and therefore are better suited for detecting subtle and spatially distributed patterns of alteration. The vast majority of multivariate machine learning studies of psychiatric disorders published so far have been limited to categorical decisions such as whether an individual belongs to a patient or control group; whether an individual will respond to treatment or not; or whether an individual will develop a disorder or not (35). Within this context, studies of the UHR population employing multivariate machine learning methods have typically focused on prediction of clinical outcome in terms of transition/non-transition to psychosis. For example, Koutsouleris and colleagues (37) demonstrated that a distributed network of abnormalities in GM volume allows prediction of subsequent transition to psychosis with an accuracy of 82% (37). This notable finding was replicated in an independent cohort by a subsequent investigation (38). However, follow-up studies of individuals at UHR have shown substantial heterogeneity in symptom progression both among those who develop psychosis and those who do not (39, 40). For instance, a recent investigation showed that about 75% of those individuals who do not develop psychosis present with symptoms remission after 3 years while the remaining 25% are still showing sub-threshold symptoms (40). In addition, even those individuals at UHR who show full or partial

remission of positive symptoms remain at a lower level of functioning compared to non-psychiatric comparison individuals (41). Another study reported that only 30% of those individuals who do not develop psychosis experience a full symptomatic and functional recovery (42). Despite the high degree of heterogeneity in clinical outcome beyond and above transition of psychosis, none of the multivariate machine learning studies of the UHR population published so far have focused on quantitative changes in symptomatology.

Here we sought to expand the existing literature by investigating the potential of structural MRI for predicting the course of clinical symptomatology at 2-year follow-up in individuals at ultra-high risk for psychosis using Relevance Vector Regression (RVR) (43). The advantage of RVR relative to other multivariate machine learning techniques, such as Support Vector Machine (35), is that it allows the quantitative prediction of a variable of interest (e.g., a patient's score on a clinical scale) at individual level, without the need for a discrete categorical decision (e.g., patients vs. controls). In recent years, RVR has been successfully used in several neuroimaging studies of healthy people (44, 45) and patients with psychiatric (46, 47) or neurological disorders (48). We therefore hypothesized that the application of RVR to neuroanatomical data, particularly GM volume and CT, would allow quantitative prediction of symptom progression at individual level with statistically significant accuracy.

MATERIALS AND METHODS

SUBJECTS

The total sample consisted of 40 subjects at ultra-high risk for psychosis (UHR), recruited at first presentation from consecutive referrals to the Outreach and Support in South London (OASIS) service in London, UK (49). OASIS is a clinical service located in Lambeth, South London, that offers treatment to individuals between 14 and 35 years of age who meet the ultra-high risk criteria for psychosis. Individuals at ultra-high risk for psychosis were identified based on the Personal Assessment and Crisis Evaluation (PACE) criteria (50).

Subsequent to MRI scanning, the UHR subjects were monitored for at least 2 years. Over the 2-year follow-up, 7 UHR individuals developed psychosis (UHR-T) and the remaining 33 did not (UHR-NT). Transition to psychosis during the follow-up period was established according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria based on clinical consensus between at least two experienced psychiatrists. Most of the UHR group (31/44; 70%) were naïve to antipsychotics at the time of scanning; the remaining 13 (30%) had been exposed to antipsychotics for an average of 9.7 weeks (SD = 13.3).

SOCIO-DEMOGRAPHIC AND CLINICAL MEASURES

Socio-demographic measures included age, gender, and years of education. Clinical symptoms were assessed in all participants at the time of scanning and at 2-year follow-up using the Positive and Negative Syndrome Scale (PANSS) (51). Symptoms in the UHR participants were also assessed using the Comprehensive Assessment of At-Risk Mental States (CAARMS) (50). Socio-demographic and clinical variables were analyzed using Student's *t*-test for continuous data and a chi square test for ordinal data. These statistical analyses were performed using the Statistical Package for the Social Sciences 19.0 (SPSS 19.0 for Windows, Chicago, IL, USA).

ACQUISITION OF NEUROANATOMICAL DATA

Neuroanatomical images were acquired using a 1.5-T GE NV/I Signa LX Horizon system (General Electric, Milwaukee, WI, USA) at the Center for Neuroimaging Sciences, King's College London. T1-weighted Inversion Recovery Spoiled Gradient structural images were acquired with the following acquisition parameters: TE = 5200 ms, TR = 15900 ms, flip angle = 20°, field of view = 220 mm × 176 mm, slice thickness = 1.5 mm, number of slices = 124, image matrix = 256 × 256 × 124.

ANALYSIS OF NEUROANATOMICAL DATA

The analysis of the MRI data comprised of three main components. Firstly, the unified segmentation procedure (52) implemented in SPM8[1] was used to segment all the images into GM, WM, and cerebrospinal fluid (CSF) partitions. We then preprocessed the images using two alternative approaches that allowed us to extract information on GM volume and CT respectively. Secondly, we used multivariate RVR (43) as implemented in the Pattern Recognition for Neuroimaging Toolbox[2] (PRoNTo). Thirdly, we performed a standard univariate analysis as implemented in Statistical Parametric Mapping (SPM8) software. Below we describe each component in more detail.

Creation of voxel-based gray matter volume maps

A fast diffeomorphic image registration algorithm (DARTEL) was used to warp the GM partitions into a new study-specific reference space with an isotropic spatial resolution of 1.5 mm^3 (53–55). The warped GM partitions were then affine transformed into the MNI space. An additional "modulation" step (56) was used to scale the GM probability values by the Jacobian determinants of the deformations to ensure that the total amount of GM in each voxel was conserved after the registration. As a final step the GM probability values were smoothed using a 8-mm FWHM Gaussian kernel.

Creation of voxel-based cortical thickness maps

A voxel-based Laplacian method (57, 58) was used to create a voxel-based cortical thickness (VBCT) map for each subject using the GM, WM, and CSF partitions created in the segmentation step. The resulting VBCT maps contained CT values within voxels identified as GM and zeros outside the cortex and were saved in the native space of the input images (0.5 mm^3 resolution). Each VBCT map was warped into the new DARTEL reference space by applying the corresponding subject-specific deformation field and resampled to an isotropic voxel size of 1.5 mm^3. The warped images were then scaled by the Jacobian determinant of the deformation and smoothed with a 6-mm Gaussian kernel. The same warps, modulation and smoothing were also applied to a binary mask created from each original VBCT map. Subsequently the warped, scaled, and smoothed VBCT maps were divided by the

[1] http://www.fil.ion.ucl.ac.uk/spm
[2] http://www.mlnl.cs.ucl.ac.uk/pronto/

corresponding warped, scaled, and smoothed mask. The effect of this procedure was to project the Gaussian smoothing kernel applied to the warped images, into the native space of the subject while preserving the CT value over a region the size of the smoothing kernel.

Multivariate RVR analyses

We examined the relationship between brain structure and changes in PANSS total score from baseline to 2 years follow-up using multivariate RVR as implemented in PRoNTo (see text footnote 2) running under Matlab (Mathworks, 2010 release) (59). This method has been described elsewhere (47). In brief, RVR is a sparse kernel learning multivariate regression method set in a fully probabilistic Bayesian framework. Under this framework, a zero-mean Gaussian prior is introduced over the model weights, governed by a set of hyperparameters – one for each weight. The most probable values for these hyperparameters are then iteratively estimated from the training data, with sparseness achieved due to the posterior distributions of many of the weights peaking sharply around zero; those training vectors associated with non-zero weights are referred to as "relevance" vectors. The optimized posterior distribution over the weights can then be used to predict the target value (e.g., PANSS score) for a previously unseen input vector (e.g., CT map) by computing the predictive distribution [for a more in-depth and detailed description see Tipping (43)].

In the current study, the input vectors (i.e., each subjects CT map) were mean centered using the training data, and an estimate for the model's generalizability obtained via leave-one-out cross validation, indexed using the Pearson correlation coefficient and mean square error (MSE) between actual and predicted difference between baseline and follow-up on PANSS total scores. The significance of both the correlation coefficient and the MSE score was estimated using a permutation test whereby the input-target data were randomly paired and the RVR re-run 1000 times. This created a distribution of correlation and MSE values reflecting the null hypothesis that the model did not exceed chance. The number of times the permuted value was greater than (or with respect to MSE values, less than), or equal to, the true value, was then divided by 1000 providing an estimated p-value for both the correlation coefficient and observed MSE. For ease of visualization, a table was also created using an arbitrary 70% threshold for all successful RVR derived weight maps, showing those regions with weight vector values in the upper, and lower, 30% of the absolute maximum weight vector values across all regions. These values represent the relative contribution of each voxel to the regression function, in the context of every other voxel.

Univariate SPM analyses

We also examined the relationship between brain structure and changes in PANSS total score from baseline to follow-up using a standard, univariate approach. A multiple regression model was performed in SPM8 software to identify any voxels in the GM volume and CT maps respectively that showed a significant association with PANSS total scores. Statistical inferences were made at $p < 0.05$ [corrected for multiple comparisons using Family-Wise Error (FWE)]. For completeness, when no significant effects were found, we also examined trends at $p < 0.001$ uncorrected.

RESULTS

SOCIO-DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

Socio-demographic and clinical variables are reported in **Table 1** for all participants as well as for the sub-groups that did and did not make transition to psychosis separately. It can be seen that, on average, participants showed clinical improvement at follow-up relative to baseline ($t = -2.555$; $p = 0.015$; df = 39). Examination of the subject-specific scores revealed that 26 individuals improved, 3 remained stable, and 11 worsened over the 2-years follow-up time. No significant association were found between the change in PANSS total scores from baseline to follow-up and antipsychotic medication ($t = -0.269$, df = 38, $p = 0.789$).

MULTIVARIATE RVR ANALYSIS

The application of RVR to whole-brain CT images allowed quantitative prediction of symptom progression with statistically significant accuracy (correlation = 0.34, p-value = 0.026; Mean Squared-Error = 249.63, p-value = 0.024, see **Figure 1**). The use of an arbitrary threshold corresponding to the top, or bottom, 30% of the maximum weight vector score showed that the prediction appeared to be based on a distributed pattern of CT including, in particular, the left insular cortex and lateral and medial regions of the right temporal cortex (see **Table 2**; **Figure 1**). In contrast, the application of RVR to the whole-brain GM volume images did not allow accurate prediction of symptoms

Table 1 | Demographic and clinical variables by group.

	Groups			Group comparison
	UHR (*N* = 40)	**UHR-NT (*N* = 33)**	**UHR-T (*N* = 7)**	
Age (years)	23.90 (4.50)	24.06 (4.61)	23.14 (4.18)	$t = 0.48$, $p = 0.63$ df = 38
N male/female	25/15	20/13	5/2	$\chi^2 = 0.29$, $p = 0.59$
Years of education	12.82 (2.31)	12.88 (2.31)	12.50 (2.51)	$t = 0.36$, $p = 0.72$, df = 36
PANSS total baseline	53.30 (14.95)	50.27 (12.02)	67.57 (19.85)	$t = -3.06$, $p = 0.004$, df = 38
PANSS total follow-up	46.50 (13.34)	43.61 (10.25)	60.14 (18.27)	$t = -3.34$, $p = 0.002$, df = 38
Difference PANSS follow-up – baseline	−6.80 (16.83); $t = -2.555$; $p = 0.015$; df = 39	6.67 (15.16)	7.43 (24.78)	$t = -0.10$, $p = 0.91$, df = 38

Data reflect mean (and standard deviation). df, Degrees of freedom; PANSS, positive and negative syndrome scale.

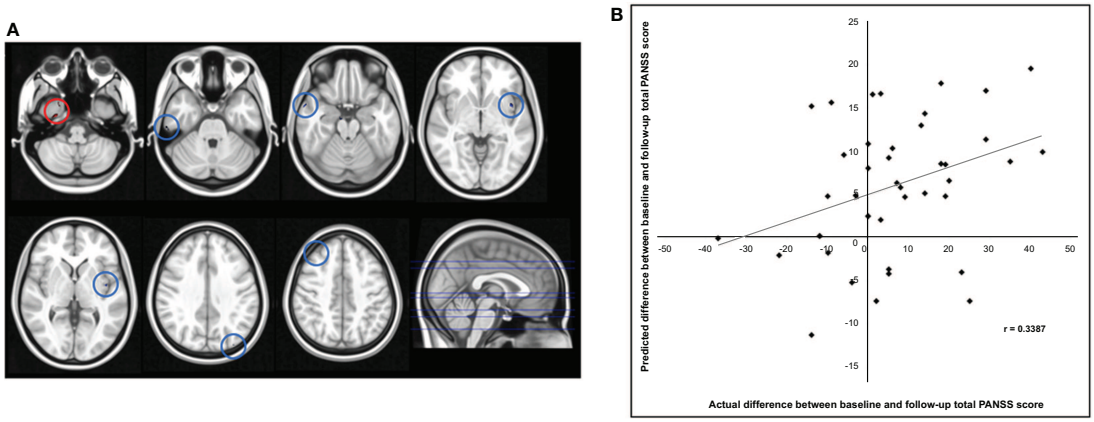

FIGURE 1 | (A) Red/Blue circles show voxels with a weight score in the top/bottom 30% of the maximum (range −0.011608 to −0.026588). Axial Slices (MNI) Left-Right: −65, −49, −40, −23, −17, 16, 25.

(B) Scatter plot showing the predicted difference between baseline and follow-up total PANSS score for each subject derived from their cortical thickness data using RVR vs. the actual difference.

Table 2 | Neuroanatomical regions with a weight vector score in the top and in the bottom 30% of the maximum weight vector score across all regions for the cortical thickness based RVR used to accurately predict the difference between baseline and follow up total PANSS score.

Region	Number of voxels	MNI coordinate (*x, y, z*)	w_i
REGIONS WITH POSITIVE w_i SCORES			
Right Temporal Fusiform Cortex	27	28.5, −9, −46.5	0.0266
Right Temporal Pole	8	25.5, 6, −48	0.0213
REGIONS WITH NEGATIVE w_i SCORES			
Right Temporal Pole	29	58.5, 6, −22.5	0.0115
Left Insular Cortex	26	−39, 6, −4.5	0.0112
	20	−42, −6, −1.4	0.0116
Right Parahippocampal Gyrus (anterior division)	9	10.5, −12, −21	0.00954
Right Inferior Temporal Gyrus (posterior division)	7	60, −27, −30	0.00882

MNI, Montreal Neurological Institute; RVR, relevance rector regression; PANSS, positive and negative syndrome scale; $\mathbf{w_i}$*, weight vector score indicating the relative contribution of each voxel to the regression function.*

$\mathbf{w_i}$ *and MNI coordinates refer to the peak weight vector score in each cluster.*

progression (correlation = 0.14, p-value = 0.627; Mean Sum of Squares = 369.50, p-value = 0.621).

UNIVARIATE SPM ANALYSIS

Whole-brain analysis of the GM volume and CT data did not detect any regions that showed a significant positive or negative association with the change in PANSS total scores from baseline to follow-up at $p < 0.05$ (FWE corrected). With a less conservative statistical threshold ($p < 0.001$ uncorrected), we detected a number of regions showing a positive association with the change in PANSS total scores. With respect to GM volume, the right middle frontal gyrus (MNI coordinates: 39, 15, 37.5; $p = 0.929$; z-score = 3.266) was associated with changes in PANSS scores. With respect to CT, the right inferior parietal lobule (MNI coordinates: 61.5, −34.5, 25.5; $p = 0.802$; z-score = 3.659), left cingulate gyrus (MNI coordinates: −9, 1.5, 46.5; $p = 0.986$; z-score = 3.340), right middle temporal gyrus (MNI coordinates: 49.5, −63, 4.5; $p = 0.992$; z-score = 3.295) and left insula (MNI coordinates: −34.5, −15, 16.5; $p = 0.998$; z-score = 3.197) were associated with changes in PANSS scores.

DISCUSSION

At present it is difficult to use clinical data acquired at first clinical presentation to predict subsequent progression of symptoms in individuals at UHR for psychosis. This prevents the selective delivery of potentially preventative interventions to those who are most likely to develop persistent symptoms. Recent studies have shown that the application of multivariate machine learning methods to structural neuroimaging data allows accurate categorical prediction of which individuals at UHR will and will not

make transition to psychosis (37). However, as discussed in the introduction, within the UHR population there is a substantial heterogeneity in symptom progression above and beyond transition to psychosis (40–42). We therefore examined for the first time whether it is possible to use neuroanatomical information to make accurate quantitative predictions of symptom progression in individuals at UHR. Our results indicate that the application of RVR to whole-brain CT MRI data allows quantitative prediction of symptom progression (i.e., both the magnitude and direction of change for each individual) at 2-year follow-up with statistically significant accuracy. To our knowledge, this is the first evidence that neuroimaging techniques may inform the clinical assessment of UHR individuals by allowing quantitative estimation of the course of clinical psychopathology. In contrast, GM volume did not allow accurate prediction of symptoms progression at individual level despite two previous reports that this information allows categorical prediction of transition to psychosis (37, 38).

What is the implication of our differential finding for CT and GM volume? GM volume is thought to depend on local CT as well as cortical folding and gyrification (i.e., cortical surface area), while CT does not include measures of local surface (57). A recent investigation has also shown that CT and cortical surface area are genetically and phenotypically independent, and that regional GM volume is more closely related to the latter than the former (60). It follows that the two approaches provide complementary information, and that one can be more or less than the other depending on the nature of the neuroanatomical changes being examined. In the context of our investigation, the fact that symptom progression was predicted by CT but not GM volume indicates that changes in symptomatology are specifically associated with differences in CT as opposed to cortical folding and gyrification.

Examination of the regions that provided the greatest contribution to prediction of symptom progression identified specific areas amongst others traditionally associated with schizophrenia, namely the right temporal fusiform cortex, the right temporal pole, the right parahippocampal gyrus, the inferior temporal gyrus, and the left insular cortex (see **Table 2**; **Figure 1**). The temporal fusiform cortex and temporal pole have been reported to show CT differences over time between UHR-T and controls but not between UHR-NT and controls (26). The temporal pole is thought to be implicated in different cognitive functions such as emotion, attention, behavior, and memory (61). In people with schizophrenia, abnormalities in this region have been associated with a range of clinical symptoms including, amongst others, auditory hallucinations and thought disorder (62, 63). The temporal fusiform cortex plays a central role in facial configuration processing in the healthy brain (64). Deficits in this domain have been recently reported in the UHR population, and may be one of the factors that underlie social dysfunction in schizophrenia (65). The parahippocampal gyrus has also been reported to show reduced thickness both in the UHR (20, 66) and first episode psychosis (67). Specifically, this area has been identified as a site of robust structural and functional alteration in individuals at ultra-high risk for psychosis (68) and those who have developed the disorder (69, 70). The right inferior temporal gyrus volume has also been reported as progressively reduced overtime in UHR-T compared to UHR-NT (2). Finally the left insular cortex plays a key role in emotional regulation, which is typically altered in psychosis, and has been found to show reduced volume in UHR-T compared to UHR-NT (2, 5).

While the results of our investigation provide further evidence for the implication of the above regions in schizophrenia, it should be noted that in multivariate methods an individual region may display high discriminative power due to two possible reasons: (i) a difference in volume between groups in that region; (ii) a difference in the correlation between that region and other areas between groups. Thus, the regions identified in our investigation should be interpreted as parts of a spatially distributed pattern rather than as independent areas. In addition, it should be noted that these regions were identified using an arbitrary threshold of 30% based on previous studies (71, 72), and that prediction of symptom progression was to some extent informed by all voxels in the brain since no feature extraction was employed.

In contrast to the results obtained using RVR, the univariate analysis of the structural MRI data, in which each voxel is considered as a spatially independent unit, did not detect any regions that showed a significant association with progression of symptoms after correction for multiple comparisons. This supports the idea that multivariate methods such as RVR are more sensitive to the subtle and spatially diffuse alterations typically observed in psychiatric disorders, and therefore may be better suited to the possible development of clinical diagnostic tools, than standard mass-univariate techniques (73).

The present study has four main limitations. Firstly, the number of subjects included in the study was relatively small and therefore the generalizability of the results is unclear. Multi-center studies would be needed in order to better characterize the predictive value of structural neuroimaging for predicting symptom progression in real-life clinical practice. Secondly, 30% of our participants had been exposed to antipsychotic medication which might have influenced our results for instance by resulting in changes in brain structure while also influencing symptom progression. Nevertheless, as we report in the Results, we found no evidence for an association between antipsychotic medication at first clinical presentation (i.e., yes/no) and progression of illness. Thirdly, there are a number of potential sources of individual variability in symptom progression that were not included in our statistical model; these include, for example, socio-demographic variables such as age, gender, and ethnicity, and treatment course variables such as life events and psychosocial interventions during the follow-up period. We expect that the integration of this information within the same statistical model would improve prediction of symptom progression. Fourthly, in the present investigation we examined the predictive value of gray rather than WM as the former could be estimated more accurately than the latter. However, given the number of studies reporting an association between WM integrity and clinical outcome in the UHR population (26, 27, 30–32), it would be interesting to use DTI scans in future studies.

In conclusion, the results of the present study provide proof-of-concept that it might be possible to use structural neuroimaging to inform quantitative prediction of subsequent progression of symptoms in individuals at UHR for psychosis. This would enable clinicians to target those individuals at greatest need of preventative interventions thereby resulting in a more efficient use of

health care resources. It should be noted, however, that daily clinical practice often requires clinicians to make prompt treatment decisions, and delaying the decisional process in order to acquire and analyze structural neuroimaging data could be impractical and potentially harmful to a patient. A possible solution would be the development of a practical and flexible analytical tool for clinical use that does not require the manual implementation of a lengthy pipeline. In addition, it is likely that the use of structural neuroimaging in everyday clinical practice would ultimately require a greater accuracy of prediction than that found in the present study. Such accuracy might be improved, for example, by combining structural neuroimaging with other types of data, an integrative approach which was successfully applied to an investigation of mild cognitive impairment (74).

AUTHOR CONTRIBUTIONS

Stefania Tognin performed the analyses and prepared the first draft of the manuscript; William Pettersson-Yeo assisted with data analyses and prepared the first draft of figure and tables. Isabel Valli and James Woolley contributed with data collection; Chloe Hutton provided the VBCT toolbox and assisted with data analyses. Andrea Mechelli designed the research study. All the co-authors contributed to the critical revision of the manuscript.

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Appendix 2. UHR clinical scores per site

Table A2.1. Clinical Measures London site (Institute of Psychiatry, Psychology and Neuroscience)

Measures	UHR-NT (n=19) Mean (±SD)	UHR-T (n=2) Mean (±SD)	Significance
CAARMS Abnormalities of Thought Content	4.16 ± 1.67	2.50 ± 3.53	$p = 0.236$
CAARMS Perceptual Abnormalities	2.68 ± 1.88	2.50 ± 3.53	$p = 0.903$
CAARMS Speech Abnormalities	1.26 ± 1.85	2.50 ± 3.53	$p = 0.410$
PANSS_Negative	9.42 ± 3.43	7 ± 0.60	$p = 0.343$
PANSS_Positive	12.32 ± 3.30	7.50 ± 0.70	$p = 0.058$
PANSS General	22.21 ± 4.09	18.50 ± 3.53	$p = 0.234$
HAM–D	10.11 ± 9.02	8 ± 8.48	$p = 0.756$
HAM-A	12.11 ± 11.62	7 ± 9.89	$p = 0.559$
GAF	62.89 ± 14.80	74 ± 12.72	$p = 0.322$
Premorbid IQ (NART)	96 ± 14.04	82 ± 6.36	$p = 0.187$

Abbreviations: UHR-T, ultra high risk with disease transition; UHR-NT, ultra high risk without disease transition; CAARMS, Comprehensive Assessment of At-Risk Mental State; PANSS, Positive and Negative Symptom Scale; HAM-D, Hamilton Depression Rating Scale; HAM-A, Hamilton Anxiety Rating Scale; GAF, Global Assessment of Functioning; NART, National Adult Reading Test.

Table A2.2. Clinical Measures London site (Maudslay Hospital)

Measures	UHR-NT (n=44) Mean (±SD)	UHR-T (n=12) Mean (±SD)	Significance
CAARMS Abnormalities of Thought Content	3.48 ± 1.38	3.50 ± 1.06	$p = 0.964$
CAARMS Perceptual Abnormalities	2.50 ± 1.90	2.75 ± 2.31	$p = 0.743$
CAARMS Speech Abnormalities	1.71 ± 1.45	3 ± 1.69	$p = 0.030$
PANSS_Negative	12 ± 4.46	14.63 ± 5.63	$p = 0.150$
PANSS_Positive	10.81 ± 3.9	11.25 ± 4.26	$p = 0.775$
PANSS_General	25.48 ± 8.15	30.25 ± 11.67	$p = 0.159$
GAF	58.12 ± 10.15	46.50 ± 10.85	$p = 0.005$
IQ (WAIS)*	99 ± 11.49	94 ± 7.47	$p = 0.241$

* Information was available for a subset of the whole sample (i.e. UHR-NT=39; UHR-T=8).

Abbreviations: UHR-T, ultra high risk with disease transition; UHR-NT, ultra high risk without disease transition; CAARMS, Comprehensive Assessment of At-Risk Mental State; PANSS, Positive and Negative Symptom Scale; GAF, Global Assessment of Functioning; WAIS, Wechsler Adult Intelligence Scale.

Table A2.3. Clinical Measures Basel site

Measures	**UHR-NT (n=23) Mean (±SD)**	**UHR-T (n=12) Mean (±SD)**	**Significance**
BPRS	37.09 ± 6.84	40.83 ± 11.45	$p = 0.233$
SANS	7.04 ± 4.50	9.75 ± 5.81	$p = 0.137$
IQ (LPS)*	111 ± 16.92	115 ± 11.57	$p = 0.550$
IQ (MWT)**	106 ± 15.76	112 ± 11.59	$p = 0.260$

* Information was available for a subset of the whole sample (i.e. UHR-NT=19; UHR-T=12)

** Information was available for a subset of the whole sample (i.e. UHR-NT=18; UHR-T=12).

Abbreviations: UHR-T, ultra high risk with disease transition; UHR-NT, ultra high risk without disease transition; BPRS, Brief Psychiatric Rating Scale; SANS, Scale for the Assessment of Negative Symptoms; LPS, German scale for assessing non-verbal IQ; MWT, German scale for assessing verbal IQ.

Table A2.3. Clinical Measures Melbourne site

Measures	**UHR-NT (n=26) Mean (±SD)**	**UHR-T (n=10) Mean (±SD)**	**Significance**
BPRS	25.52 ± 11.86	25.33 ± 10.97	$p = 0.967$
SANS	31.93 ± 18.32	32.78 ± 13.59	$p = 0.899$
Premorbid IQ (NART)*	91 ± 11.17	98 ± 10.32	$p = 0.175$

* Information was available for a subset of the whole sample (i.e. UHR-NT=17; UHR-T=6)

Abbreviations: UHR-T, ultra high risk with disease transition; UHR-NT, ultra high risk without disease transition; BPRS, Brief Psychiatric Rating Scale; SANS, Scale for the Assessment of Negative Symptoms; NART, National Adult Reading Test.

Table A2.4. Clinical Measures Munich site

Measures	**UHR-NT (n=24) Mean (±SD)**	**UHR-T (n=16) Mean (±SD)**	**Significance**
PANSS_Negative*	12.05 ± 5.15	16.25 ± 8.97	$p = 0.136$
PANSS_Positive*	9.74 ± 2.28	12.88 ± 4.45	$p = 0.022$
PANSS_General*	28.42 ± 6.34	29.25 ± 11.14	$p = 0.209$
IQ (MWT)**	111 ± 13.87	104 ± 17.22	$p = 0.226$

* Information was available for a subset of the whole sample (i.e. UHR-NT=19; UHR-T=8)
** Information was available for a subset of the whole sample (i.e. UHR-NT=18; UHR-T=11)
Abbreviations: UHR-T, ultra high risk with disease transition; UHR-NT, ultra high risk without disease transition; PANSS, Positive and Negative Symptom Scale; MWT, German scale for assessing verbal IQ.

Appendix 3. Sociodemographic data of the study samples by site

A3.1. Sociodemographic data of the study samples included in the study described in Chapter 3

Group	Characteristics	London (Institute of Psychiatry)	London (Maudsley Hospital)	Basel	Munich	Melbourne	Significance
UHR-NT	**Number (%)**	19 (90.5%)	42 (84%)	23 (65.7%)	24 (60%)	26 (72.2%)	$\chi^2_4 = 10.831$ $p = 0.029$
	Age (mean/ SD)	23.47 ± 4.89	23.76 ± 4.64	23.26 ± 5.8	26.08 ± 6.11	20.04 ± 3.06	$F_{(4,129)} = 4.83$ $p = 0.01$
	Gender (Female/Male)	8/11	16/26	10/13	11/13	11/15	$\chi^2_4 = 0.428$ $p = 0.98$
	Ethnicity Caucasian Black Asian Mixed	 11 3 1 4	 25 4 2 11	 23 0 0 0	 24 0 0 0	 23 0 3 0	$\chi^2_{12} = 38.651$ $p < 0.01$
	Handedness Right Left Ambidextrous	 18 1 0	 41 1 0	 22 1 0	 20 2 2	 25 1 0	$\chi^2_8 = 10.796$ $p = 0.214$
	GMV (mean, SD)	1.004 ± 0.137	0.928 ± 0.08	0.881 ± 0.09	0.921 ± 0.07	0.96 ± 0.08	$F_{(4,129)} = 5.126$ $p = 0.01$
UHR-T	**Number (%)**	2 (9.5%)	8 (16%)	12 (34.3%)	16 (40%)	10 (27%)	$\chi^2_4 = 10.831$ $p = 0.029$ * $\chi^2_3 = 7.003$ $p = 0.072$

	Age (mean, SD)	27.5 ± 2.5	21.88 ± 2.47	24.58 ± 5.26	23.38 ± 4.55	19.20 ± 2.61	* $F_{(3,42)} = 3.423$ $p = 0.026$
	Gender (Female/Male)	2/0	1/7	3/9	4/12	4/6	$\chi^2_3 = 1.787$ $p = 0.618$
	Ethnicity Caucasian Black Asian Mixed	 1 0 1 0	 2 1 0 5	 12 0 0 0	 16 0 0 0	 10 0 0 0	$\chi^2_6 = 32.775$ $p < 0.01$
	Handedness Right Left Ambidextrous	 2 0 0	 7 0 1	 9 3 0	 16 0 0	 10 0 0	$\chi^2_8 = 14.591$ $p = 0.068$
	GMV (mean, SD)	0.925 ± 0.088	0.916 ± 0.113	0.909 ± 0.068	0.976 ± 0.073	0.969 ± 0.094	* $F_{(3,42)} = 1.988$ $p = 0.13$
Healthy Controls	**Number**	27	37	22	42	39	n/a
	Age (mean/ SD)	24.93 ± 3.85	25.38 ± 4.51	23 ± 4.3	24.48 ± 3.78	20.1 ± 2.34	$F_{(4,162)} = 11.956$ $p < 0.01$
	Gender (Female/Male)	14/13	10/27	9/13	14/28	14/25	$\chi^2_4 = 4.549$ $p = 0.337$
	Ethnicity Caucasian Black Asian Mixed	 12 9 5 1	 25 2 5 5	 22 0 0 0	 42 0 0 0	 39 0 0 0	$\chi^2_{12} = 76.146$ $p < 0.01$
	Handedness Right Left Ambidextrous	 26 1 0	 35 0 2	 16 5 1	 35 5 2	 36 2 1	$\chi^2_8 = 13.824$ $p = 0.86$

	GMV (mean, SD)	0.972 ± 0.194	0.915 ± 0.089	0.902 ± 0.066	0.926 ± 0.091	1.009 ± 0.089	$F_{(4,162)} = 5.46$ $p < 0.01$

* Analysis performed by excluding the London (Institute of Psychiatry) site.
Abbreviations: UHR-T, ultra high risk with disease transition; UHR-NT, ultra high risk without disease transition.

A3.2. Sociodemographic data of the study samples included in the studies described in Chapter 4 and Chapter 5

Group	Characteristics	London (Maudsley Hospital)	Basel	Munich	Melbourne	Significance
UHR-NT	**Number (%)**	44 (78.6%)	23 (65.7%)	24 (60%)	26 (72.2%)	$\chi^2_3 = 4.259$ $p = 0.235$
	Age (mean/ SD)	24.14 ± 4.9	23.26 ± 5.8	26.08 ± 6.11	20.04 ± 3.06	$F_{(3,113)} = 6.442$ $p < 0.01$
	Gender (Female/Male)	17/27	10/13	11/13	11/15	$\chi^2_3 = 0.370$ $p = 0.946$
	Ethnicity Caucasian Black Asian Mixed	 26 5 2 11	 23 0 0 0	 24 0 0 0	 23 0 3 0	$\chi^2_9 = 36.575$ $p < 0.01$
	Handedness Right Left Ambidextrous	 43 1 0	 22 1 0	 20 2 2	 25 1 0	$\chi^2_8 = 11.486$ $p = 0.074$
	GMV (mean, SD)	0.906 ± 0.09	0.881 ± 0.09	0.921 ± 0.07	0.96 ± 0.08	$F_{(3,113)} = 3.745$ $p = 0.013$
UHR-T	**Number (%)**	12 (21.4%)	12 (34.3%)	16 (40%)	10 (27%)	$\chi^2_3 = 4.259$ $p = 0.235$
	Age (mean, SD)	23.25 ± 4.11	24.58 ± 5.26	23.38 ± 4.55	19.20 ± 2.61	$F_{(3,46)} = 3.112$ $p = 0.035$
	Gender (Female/Male)	2/10	3/9	4/12	4/6	$\chi^2_3 = 1.577$ $p = 0.665$

	Ethnicity Caucasian Black Asian Mixed	 5 1 0 6	 12 0 0 0	 16 0 0 0	 10 0 0 0	$\chi^2_6 = 25.775$ $p < 0.01$
	Handedness Right Left Ambidextrous	 10 1 1	 9 3 0	 16 0 0	 10 0 0	$\chi^2_6 = 10.231$ $p = 0.115$
	GMV (mean, SD)	0.893 ± 0.13	0.909 ± 0.068	0.976 ± 0.073	0.969 ± 0.094	$F_{(3,46)} = 2.556$ $p = 0.067$
Healthy Controls	**Number**	47	22	42	39	n/a
	Age (mean/ SD)	25.51 ± 4.45	23 ± 4.3	24.48 ± 3.78	20.1 ± 2.34	$F_{(3,146)} = 15.882$ $p < 0.01$
	Gender (Female/Male)	14/33	9/13	14/28	14/25	$\chi^2_3 = 0.911$ $p = 0.823$
	Ethnicity Caucasian Black Asian Mixed	 28 7 6 6	 22 0 0 0	 42 0 0 0	 39 0 0 0	$\chi^2_9 = 41.591$ $p < 0.01$
	Handedness Right Left Ambidextrous	 45 0 2	 16 5 1	 35 5 2	 36 2 1	$\chi^2_6 = 12.303$ $p = 0.056$
	GMV (mean, SD)	0.874 ± 0.118	0.902 ± 0.066	0.926 ± 0.091	1.009 ± 0.089	$F_{(3,146)} = 14.601$ $p < 0.01$

Abbreviations: UHR-T, ultra high risk with disease transition; UHR-NT, ultra high risk without disease transition.